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Cardiac prostaglandin release during myocardial ischemia

Harvey James Berger
Yale University

CARDIAC PROSTAGLANDIN RELEASE DURING MYOCARDIAL ISCHEMIA

HARVEY JAMES BERGER

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TABLE OF CONTENTS

INTRODUCTION

Biochemistry and Metabolism of Prostaglandins

In the 1930's, prostaglandins were discovered as constituents of semen and were found to be vasodepressor compounds. Their chemical structure and relationship to the essential fatty acids were first described by Bergstrom and Van Dorp in the early 1960's. Prostaglandins have been found in almost all tissues studied, including lung, kidney, ovaries, spleen, nerves, and heart (1).

Under the influence of an enzyme complex, prostaglandin synthetase, the primary prostaglandins are synthesized from the precursor fatty acids, dihomolinolenic acid and arachidonic acid, both of which are derived in animal tissue from the essential fatty acid, linoleic acid (2).

The prostaglandins are 20 carbon compounds and are divided structurally into three major subgroups: A, E, and F. The E prostaglandins are characterized by 11-hydroxy and 9-keto groups. In contrast, the F class has a second hydroxyl group in lieu of the 9-keto group. The prostaglandins are subdivided further by the number of double bonds in the aliphatic side chains. Prostaglandins E_1 and F_1 contain a single bond at the C 13:14 position, whereas prostaglandins E_2 and F_2 possess a double bond at that position

The E_1 and F_1 prostaglandins are synthesized from dihomolenolenic acid, while E_2 and F_2 prostaglandins originate from arachidonic acid. Prostaglandin A is a dehydration product *in vivo* of prostaglandin E (Fig 1).

Using the pathway of arachidonic acid to prostaglandins E_2 and F_2 as a model, the synthetase enzyme first produces two highly active intermediates, endoperoxides G_2 and H_2. These compounds are extremely labile and rapidly converted to F_2 and E_2. In addition, metabolism of the endoperoxides results in formation of thromboxanes and another endoperoxide, D_2. It is believed that the thromboxane pathway parallels that of the primary prostaglandins and includes another enzyme complex, termed thromboxane synthetase. This pathway yields thromboxane A_2, which then is converted to B_2. These compounds are also short-lived, but very active in certain systems such as platelets (3,4).

It appears that the precursor fatty acids are stored within the phospholipids of cell membranes. When stimulated, the endoperoxides, prostaglandins, and thromboxanes are rapidly synthesized and probably stored within the membrane. Prostaglandins are rapidly inactivated by a 15-hydroxyprostaglandin dehydrogenase either at the site of action or by transport in the circulation to the lung, liver, or kidney (5-7). Arterial levels of prostaglandins E and F are very low, because these compounds are almost completely inactivated

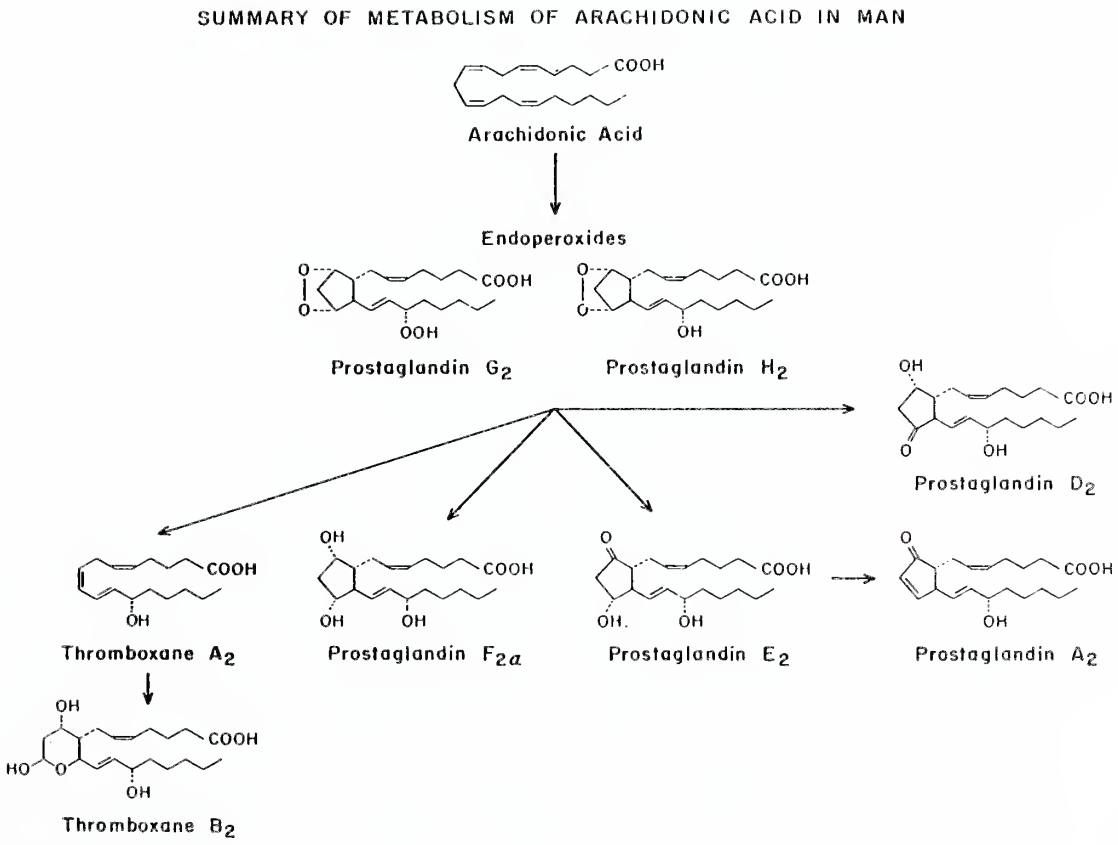

SUMMARY OF METABOLISM OF ARACHIDONIC ACID IN MAN
COOH
Arachidonic Acid
Endoperoxides
COOH
OOH
Prostaglandin G_2
COOH
OH
Prostaglandin H_2
OH
COOH
O
OH
Prostaglandin D_2
COOH
OH
Thromboxane A_2
OH
COOH
OH
OH
Prostaglandin $F_{2\alpha}$
O
COOH
OH
OH
Prostaglandin E_2
O
COOH
OH
Prostaglandin A_2
OH
COOH
HO
O
OH
Thromboxane B_2

Figure 1.

during a single circulation.

The heart is capable of synthesizing and degrading prostaglandins locally, as both the synthetase (8) and dehydrogenase (9) enzymes have been identified in canine left ventricle. Prostaglandin synthesis in vascular smooth muscle (10,11), including coronary arteries (12,13), also has been documented.

The full range of cellular actions of the prostaglandins is not known at this time. In general, the prostaglandins, especially of the E class, increase the level of intracellular cyclic AMP, probably via adenylate cyclase (14).

General Pharmacology

A potentially powerful means of elucidating the actions of these ubiquitous compounds has been inhibition of prostaglandin synthetase by anti-pyretic, anti-inflammatory, and analgesic agents, such as aspirin and indomethacin (15,16). These drugs have similar actions relative to the prostaglandin pathway, except for one intriguing difference: indomethacin blocks cardiac prostaglandin synthetase *in vitro*, while aspirin does not (8). Docosahexanoic acid, a fatty acid related to arachidonate, is also a potent inhibitor of prostaglandin synthetase (Fig 2). Other fatty acids, such as linolenate and oleate, however, are not known to be inhibitors (15).

One experimental anti-inflammatory agent has more specific inhibitory effects. This compound, benzydamine, is related structurally to indomethacin, with almost the same basic ring structure.

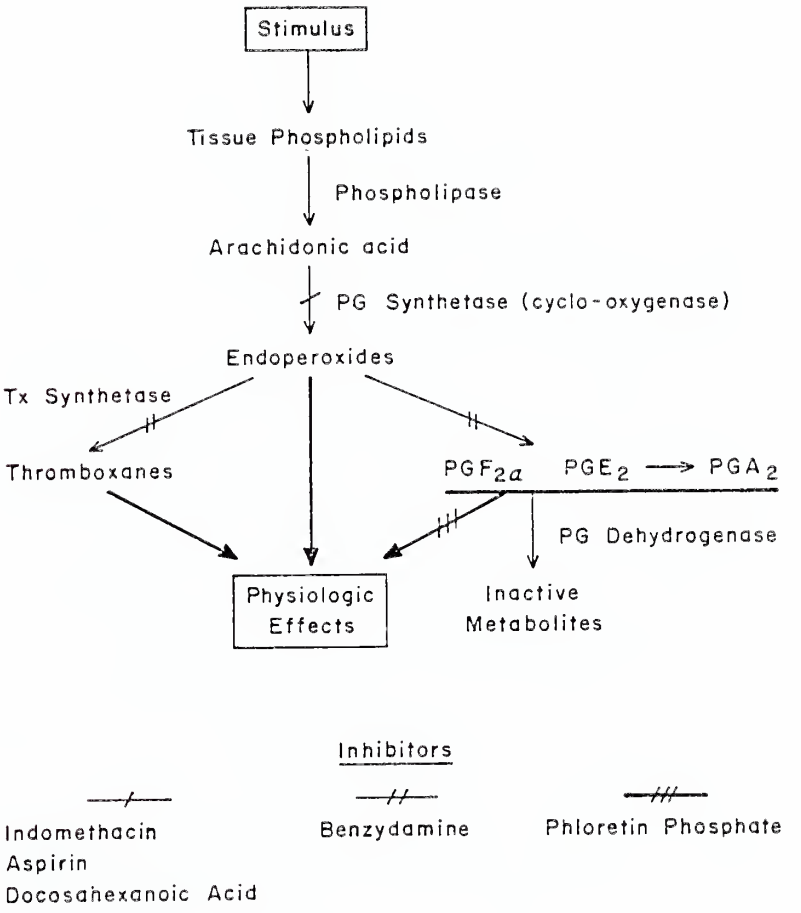


Figure 2. Prostaglandin pathways from stimulus, such as ischemia, through physiologic effects. Presumed sites of inhibition are noted by the crossed arrows.

Using an in vitro assay system, benzydamine was found to selectively inhibit synthesis of prostaglandin F and endoperoxide D, while either augmenting or not affecting prostaglandin E biosynthesis in different doses (17). In a vascular smooth muscle preparation, its inhibition of thromboxane synthetase was 2 1/2 times more effective by weight than that of prostaglandin synthetase. Of note, indomethacin's inhibition of the prostaglandin system was 10 times as potent as its inhibition of thromboxane synthetase (18).

Other unrelated compounds, such as phloretin phosphate, have been found to antagonize the end-organ effects of the prostaglandins, by acting as competitive inhibitors. These agents primarily interact with the receptor site for the agonist, rather than with the synthetase enzyme (19,20).

Actions of Exogenously Administered Prostaglandins

In both isolated hearts and intact animals, prostaglandins E and A increase coronary blood flow and decrease coronary vascular resistance (21-26). The coronary vasodilator actions of these compounds are not mediated via the adrenergic or cholinergic nervous systems, as pretreatment with propranolol or atropine does not inhibit their vasodilatory effects (21,23,24). The inotropic actions of prostaglandins E and A have not been well defined and

are dependent on the species and model studied (25-27). In the intact animal, it is generally agreed that prostaglandin E produces increased inotropy and chronotropy (Fig 3), although separation of primary and secondary effects is still not resolved fully (26,27). Some studies of prostaglandins in isolated ventricular myocardium have found no direct inotropic or chronotropic effects (25,26). When given systemically, prostaglandins E and A decrease arterial blood pressure profoundly by decreasing total peripheral vascular resistance. In contrast, prostaglandin F has little, if any, effect on blood pressure or coronary blood flow (23,24); only when given in high doses does prostaglandin F increase systemic arterial pressure, probably by peripheral venoconstriction (28).

Studies in the isolated innervated rabbit heart have shown that prostaglandin E inhibits sympathetic and parasympathetic neurotransmission, mainly by decreasing release of transmitter from nerve endings. These findings suggest that prostaglandins synthesized endogenously act as physiological modulators of neurotransmission in the heart (29).

In experimental myocardial infarction, pharmacologic doses of prostaglandins have been shown to have beneficial effects. One study showed that prostaglandin E increased coronary blood flow to the ischemic area in comparison with the preinfarction state (30). In another study, after acute coronary occlusion in the cat,

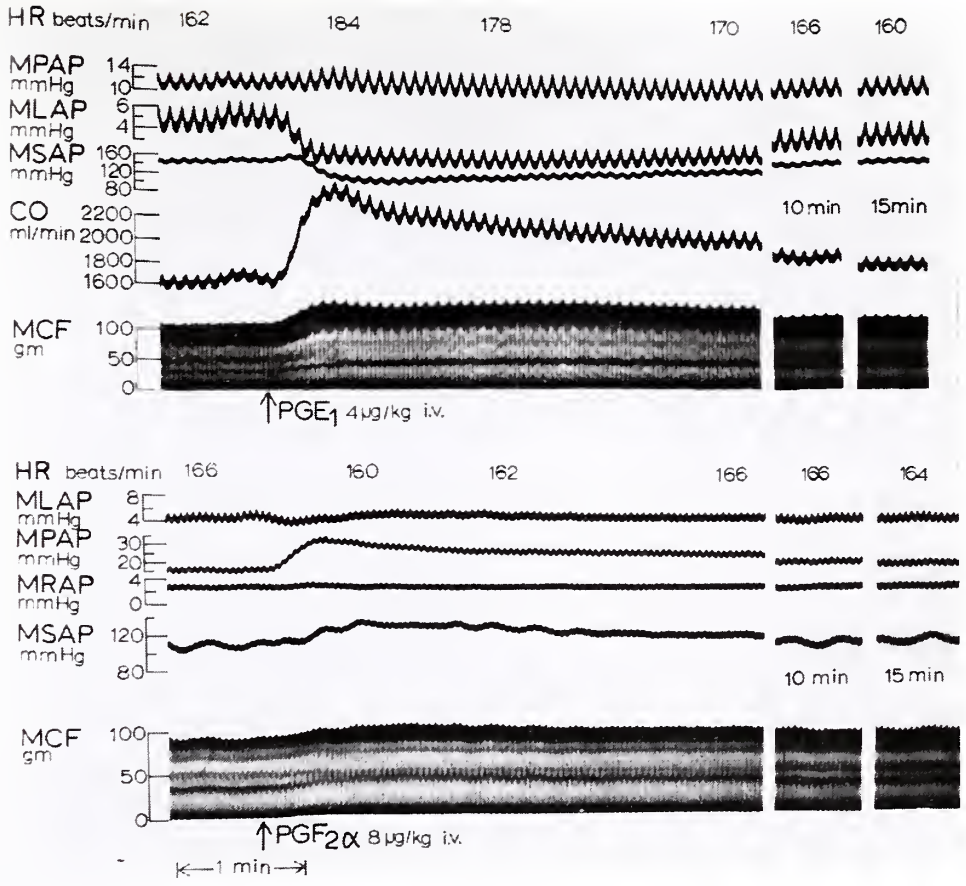


Figure 3. Hemodynamic effects of intravenous prostaglandin E_1 (PGE) and prostaglandin $F_{2\alpha}$ (PGF) in the anesthetized dog. PGE caused a rapid decrease in mean left atrial pressure (MLAP) and mean systemic arterial pressure (MSA) and a rise in heart rate (HR), cardiac output (CO), and myocardial contractile force (MCF). In contrast PGF had minimal effects on HR, MLAP, and MCF. MSAP and mean pulmonary arterial pressure (MPAP) rose slightly. (From Nakano, reference 23).

administration of prostaglandin F increased survival time, decreased plasma creatine phosphokinase, and decreased myocardial lysosomal activity compared to controls (31,32). Recent reports have suggested that release of cardiac lysosomal hydrolases may signal irreversible cell death. They also have been implicated in the development of collateral blood flow (33). Stabilization of these enzymes by a locally synthesized agent, such as prostaglandin F, may maintain cellular viability and local perfusion. The incidence of significant ventricular arrhythmias was decreased by prostaglandin E following coronary occlusion in cats (34). In a similar study in monkeys, a stable derivative of prostaglandin A enhanced recovery from ventricular arrhythmias due to coronary occlusion (35). These later studies suggest a beneficial effect of prostaglandins on electrical stability of the ischemic myocardium.

Physiologic Role of Endogenous Prostaglandins

The release of prostaglandins from tissues subjected to acute ischemia was first documented in the canine kidney (36). Prostaglandins have been implicated in vasomotor autoregulation in the kidney (37), brain (38), and uteroplacental bed (39).

Prostaglandin biosynthesis and release have been demonstrated in the isolated perfused rabbit heart exposed to hypoxia (40), mechanical massage, elevated preload (41), vagal stimulation (42),

and adenosine triphosphate (43) or acetylcholine (42) administration. Ischemia and anoxia have produced varied effects on prostaglandin biosynthesis (43-45), whereas acidosis, hyperthermia, hypothermia, hyperosmolarity, and hyperkalemia all were without effect (41). Studies utilizing the open-chest dog have shown an increase in prostaglandins in coronary venous blood during postocclusive reactive hyperemia (46). The same finding has been reported following coronary occlusion in the canine heart-lung preparation (47). Based on these findings, it has been suggested that prostaglandins may play a regulatory role in the cardiac response to ischemia.

However, not all published data support a role for prostaglandins in the regulation of coronary flow. One study has shown that indomethacin diminishes the increase in coronary blood flow that results after release of coronary occlusion (48), while another has not reproduced these results (49). In one report, indomethacin pretreatment did not alter changes in coronary blood flow induced by changes in coronary perfusion pressure (50). Furthermore, in several models, indomethacin did not affect resting coronary blood flow (45,46). During a 5 hour coronary occlusion in cats, neither indomethacin nor acetylsalicylic acid had any effect on ST segment elevation, creatine phosphokinase release, or systemic hemodynamics (51). In contrast, aspirin pretreatment in open-chest dogs markedly

reduced the extent of ischemic myocardial injury (ST segment elevation) during acute occlusion (52). Of note, in the isolated heart model, neither phloretin phosphate nor indomethacin blocked the reactive hyperemic response following occlusion (53).

Many of these conflicts may be traced to species differences or to technical factors relating to the models used. Most of the studies cited, which utilized inhibitors of prostaglandin synthesis, do not confirm that prostaglandin synthesis actually was blocked.

Since platelet aggregation in the presence of thrombin also results in prostaglandin release, this phenomenon may also be relevant in the setting of coronary occlusion (54). Of interest, prostaglandin E_1 is a very potent inhibitor of platelet aggregation, while prostaglandin E_2 stimulates aggregation and prostaglandin F is without effect. It has been known for many years that aspirin inhibits the second phase of platelet aggregation. More recently, it has been shown that aggregating platelets release large amounts of the short-lived endoperoxides, which are extremely potent inducers of aggregation and that aspirin blocks the formation of these endoperoxides (3,54).

In addition to the primary and intermediate prostaglandins, thromboxanes also have been implicated in platelet aggregation (55).

Furthermore, these compounds rapidly contract strips of porcine coronary artery (56). It was suggested that platelet aggregation in areas of damaged endothelium causes release of thromboxane A_2 and results in constriction of large coronary arteries. Prostaglandins and thromboxanes have opposite effects when studied in isolated coronary artery muscle strips (12,13). While prostaglandins E_2 and F_2 constrict these strips, their precursor, arachidonic acid, causes relaxation (12,57). The arachidonate-induced coronary relaxation was blocked by indomethacin and aspirin. Addition of indomethacin to the strips previously relaxed by arachidonate caused contraction. The fatty acids, linoleate and oleate, also caused relaxation both before and after indomethacin, thereby eliminating a direct non-specific effect of fatty acids as the cause of arachidonate-induced relaxation. These results suggest that arachidonic acid undergoes conversion within coronary vascular muscle to an endogenous dilating compound, which may be a thromboxane (12). It is puzzling to note that in this *in vitro* model, prostaglandin E causes smooth muscle contraction, while *in vivo*, it is a potent vasodilator, suggesting muscular relaxation.

Many investigators have hypothesized potentially beneficial effects for aspirin in myocardial infarction or ischemia. These have been based primarily on inhibition of the second phase of

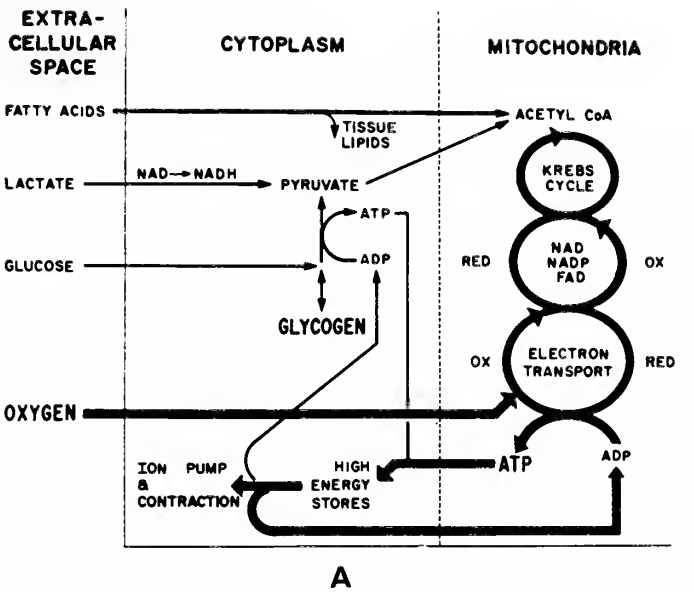
platelet aggregation (58). The clinical and experimental reports are conflicting. Aspirin (30 mg/kg, iv) has produced a favorable effect during coronary occlusion in the open-chest dog, manifested by a decrease in epicardial ST segment elevation (52). In a randomized controlled trial, chronic aspirin therapy was ineffective in reducing reinfarction in patients who had experienced an earlier myocardial infarction (59). However, the Boston Collaborative Drug Surveillance Group reported a negative association between aspirin intake and subsequent non-fatal myocardial infarction, suggesting that aspirin protects against this disease (60). One study that evaluated aspirin in patients with angina pectoris found that this drug did not affect exercise tolerance or ischemic electrocardiographic changes (61). The controversy is unresolved at this time.

Myocardial Intermediary Metabolism

The myocardial cell generates virtually all its energy by oxidative phosphorylation within the mitochondria, uses almost any substrate as fuel for this process, and functions essentially by aerobic processes under normal conditions. This is attested to by the large number of mitochondria within the myocardial cell and the extremely rich concentration of oxidative and respiratory chain enzymes.

Free fatty acids are decarboxylated, and glucose passes down the Embden-Meyerhof pathway until it is transformed into pyruvate. Each two-carbon fragment is transferred with acetyl coenzyme A from the cytoplasm to the mitochondrion where these components enter into the tricarboxylic acid cycle (Fig 4A). They they are broken down into smaller units with the progressive release of electron energy. As energy is released and captured within the various flavoproteins and oxidases, carbon dioxide is generated and high-energy phosphate compounds are synthesized, the compound richest in energy being adenosine triphosphate. Under ordinary conditions, this high-energy phosphate is available for the performance of cellular work at the membrane pumping sites, at the contractile proteins, or in cell synthesis (62,63).

This cycle is interrupted, however, whenever there is insufficient oxygen to join with free hydrogen at the end of the hydrogen accepter chain in the final formation of water (Fig 4B). During ischemia, when oxygen availability is limited, this causes a reduction in the chemical state of each of the constituents of the mitochondrial respiratory chain. When these moieties exist in reduced forms, the Krebs cycle slows down and hydrogen is transferred from the mitochondrion to the cytoplasm. The later phenomenon occurs by means of a hydrogen shuttle, presumably utilizing



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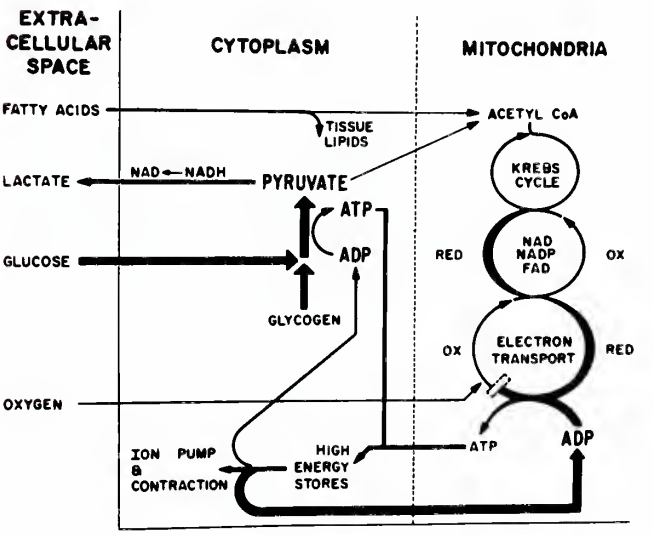

Figure 4. Myocardial intermediary metabolism.

4A (Upper): Normal pathways.

4B (Lower): Modified pathways during ischemia.

Note the production of lactate from pyruvate and its diffusion into the extracellular space. The thickness of arrows represents relative changes. (From Scheuer, reference 62).

the amino acid carnitine as the intermediate substance. As a result of this shuttle, the NAD of the cytoplasm becomes as reduced as the NAD of the mitochondrion. As soon as there is sufficient NADH in the cytoplasm, this shifts the redox potential of the lactate-pyruvate reaction.

Under normal conditions, pyruvate couples with acetyl coenzyme A to enter the mitochondrial energy cycle. Now, however, in the presence of NADH, pyruvate acts as a hydrogen acceptor and becomes lactate. Thus, pyruvate is shunted away from the mitochondria, and cytoplasmic lactic acid is generated. This lactic acid accumulates within the cell in a concentraion many times greater than that in the plasma and eventually appears within extracellular fluid and finally in the coronary capillaries and venous circulation (62,63).

Prostaglandin Substrates

The relationship of prostaglandins and free fatty acids is intriguing (2). It has been shown in the past that elevation of plasma concentrations of free fatty acids increases myocardial oxygen consumption, depresses regional myocardial contractility, and increases the incidence of arrhythmias (64,65).

Fatty acids are important fuels for myocardial cells, and

prostaglandins are known to stimulate fatty acid uptake in isolated hearts. It is not known whether the type of free fatty acid reaching the heart determines its function or influences the degree and direction of prostaglandin synthesis. If common free fatty acids act as less effective substrates for prostaglandin synthetase and thus inhibit the system, this may explain some of the deleterious effects reported for free fatty acids in the setting of myocardial ischemia (15,16).

The fatty acids that serve as precursors for prostaglandin synthesis are released from membrane phospholipids. These phospholipids also contain fatty acid derivatives that may act as inhibitors of prostaglandin synthesis, and these inhibitors may be released with the necessary precursor fatty acids (2). Prostaglandin biosynthesis and control of cellular lipolysis thus may be interrelated and depend on fatty acid composition of membrane phospholipids. For example, while arachidonic acid is a precursor of prostaglandin E_2, a structurally similar fatty acid, docosahexanoic acid, is a potent inhibitor. This is most probably accomplished by competitive binding at the substrate site of the oxygenase (synthetase) (15). In the dog, infusion of arachidonic acid results in an increase in total coronary blood flow. The investigators hypothesized that this was due to synthesis of vasodilatory prostaglandins. The increase in flow was blocked

by pretreatment with indomethacin (66). Arachidonic acid also has been shown to contract isolated smooth muscle strips, an effect blocked by indomethacin (57). Further confirmation of such pathways *in vivo* is still required.

MATERIALS AND METHODS

Studies I and II: Cardiac Prostaglandin Release During Coronary Occlusion in Closed-Chest Dogs

Initial experiments (I) were designed to determine if prostaglandins are released from the heart following coronary occlusion; which prostaglandins are released; and the time course of release. Subsequent experiments (II) investigated the site of prostaglandin release by selective sampling of venous drainage from ischemic and non-ischemic myocardial regions.

I. Seven healthy closed-chest mongrel dogs, weighing 20-31 kg, were anesthetized with sodium pentobarbital (35 mg/kg, iv) and ventilated mechanically with room air by a Harvard respirator. All animals received 3000 U heparin iv. Blood volume removed for sampling was replaced simultaneously by normal saline.

A no 7 French Sones catheter was positioned fluoroscopically approximately 2 cm beyond the orifice of the coronary sinus. Aortic pressure was measured by a Statham P23DB transducer connected through a fluid-filled system to a catheter placed in the descending aorta. The lead V_5 electrocardiogram was monitored continuously. Pressure and electrocardiogram were recorded on a DR-12 Electronics for Medicine multi-channel recorder.

Baseline aortic and coronary sinus blood samples (7 ml each) were drawn simultaneously. A balloon-tipped catheter was then positioned under fluoroscopic control in the proximal left anterior descending coronary artery. This placement was confirmed by injection of contrast medium (sodium meglumine and diatrizoate). The balloon then was inflated, completely occluding the left anterior descending coronary artery (Fig 5). ST segment elevation consistent with ischemia was noted immediately in all animals. Following occlusion, and at 5 to 10 min intervals thereafter, blood samples were drawn from the aorta and coronary sinus (7 ml each) into plastic syringes. Sampling times following occlusion differed in individual animals because of variation in the animals' hemodynamic and electrical responses to ischemia. Experiments were continued until death from ventricular arrhythmias or up to 3 hours in surviving animals.

Following occlusion, arterial pressure decreased by about 20% in every animal. None went into shock unless ventricular tachycardia or fibrillation occurred.

In 2 control animals, the identical protocol was carried out without inducing coronary occlusion. Samples were obtained from similar sites at 15 min intervals for a total of 90 min.

II. Six closed-chest dogs (22-29 kg), anesthetized with sodium

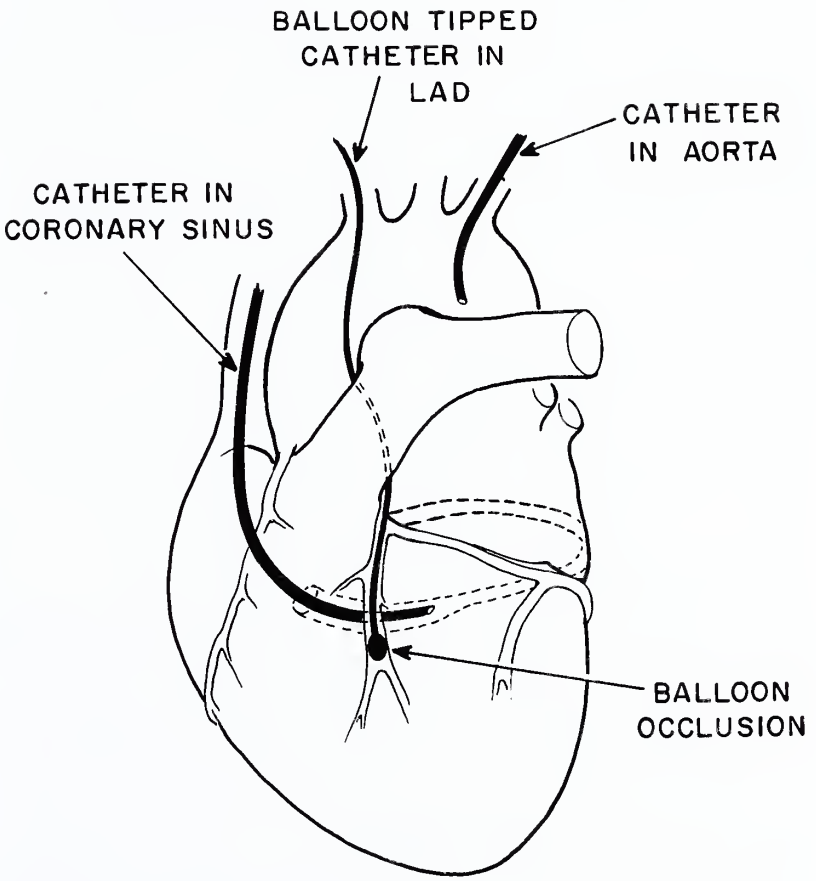

Figure 5. Experimental preparation used in Study I. Sampling catheters are in the coronary sinus and aorta. The inflated balloon tipped catheter is located in the left anterior descending (LAD) coronary artery.

pentobarbital (35 mg/kg, iv) were studied. A no 7 French Sones catheter was positioned fluoroscopically in the proximal coronary sinus 2 cm beyond the orifice; another was passed more distally into the great cardiac vein as far anteriorly as possible. Aortic pressures and electrocardiogram were monitored as previously described. A balloon-tipped catheter was placed under fluoroscopic control into the left circumflex coronary artery (Fig 6). Its position was confirmed by injection of contrast medium. Baseline samples from the aorta, coronary sinus, and great cardiac vein were obtained before inflation of the balloon and then at 5 to 10 min intervals thereafter.

The great cardiac vein receives anterior drainage from the area perfused by the left anterior descending artery, while both the anterior great cardiac vein effluent and the posterior drainage from the left circumflex distribution reach the coronary sinus (67). Therefore, during left circumflex occlusion, great cardiac vein effluent would be from normal (non-ischemic) myocardium, whereas coronary sinus drainage would necessarily include blood from ischemic and non-ischemic regions. Such sampling allows analysis of regional prostaglandin release.

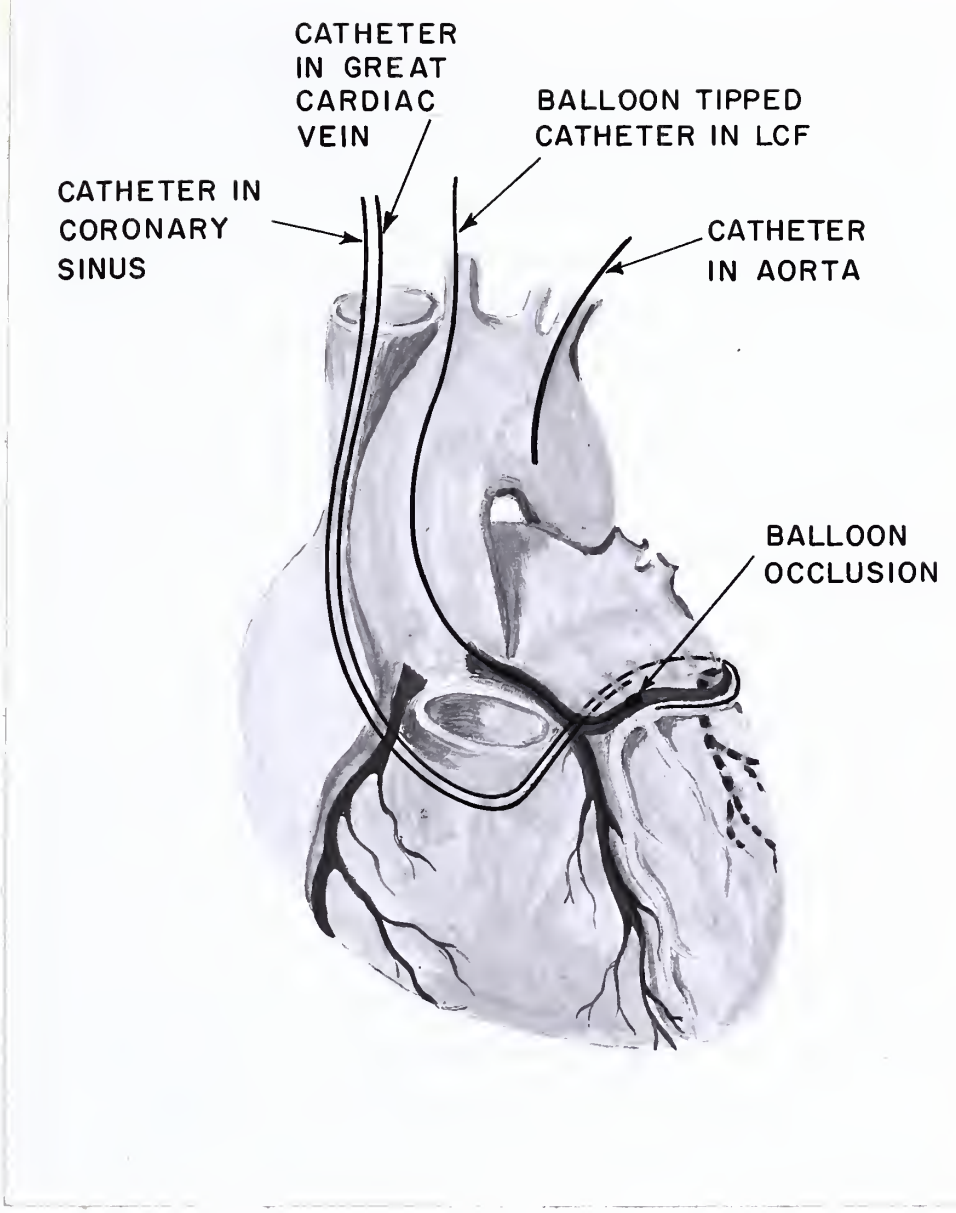

Figure 6. Experimental preparation used in Study II. The inflated balloon tipped catheter is in the left circumflex (LCF) coronary artery. Catheters in the great cardiac vein and coronary sinus are used for sampling from the non-ischemic and ischemic zones, respectively.

Data Analysis

The first sample obtained immediately after occlusion never showed any arterio-venous prostaglandin difference. This sample, therefore, was not included in analysis in any experiment. All subsequent post-occlusion samples were analyzed by several approaches. Firstly, each animal was treated as a separate, independent experiment in which paired aorta and coronary sinus, aorta and great cardiac vein, or coronary sinus and great cardiac vein samples were compared by a paired t test. In addition, for each of the three sampling sites, the mean $\pm$ SE post-occlusion value was calculated for each animal. Secondly, individual animals were grouped to assess the overall response. Because the number of samples obtained after occlusion differed in individual studies, the group means for each sampling site were determined from weighted means of each animal's prostaglandin level; the weighting factor used was the inverse of the individual variances (68). Paired, weighted aorta and coronary sinus, aorta and great cardiac vein, or coronary sinus and great cardiac vein samples were compared by a t test.

Analysis of prostaglandin levels at baseline before occlusion and at the time of maximal prostaglandin release after occlusion was also undertaken. At each of these sampling times, arterial and venous values were compared by a paired t test. Mean $\pm$ SE arterio-venous

differences at these times were also determined.

Probability (P) less than 0.05 was considered significant.

Study III: Cardiac Prostaglandin Release During Atrial Pacing in Patients with Coronary Artery Disease

This study was designed to document the presence or absence of prostaglandin release from the human heart during varifiable myocardial ischemia in patients with coronary artery disease.

Atrial pacing for evaluation of myocardial lactate metabolism during ischemia is routinely performed during diagnostic cardiac catheterization in this laboratory. This protocol was approved by the Yale University School of Medicine Human Investigation Committee, and all patients gave informed consent for participation. Twelve patients were selected for inclusion in the study. The selection criteria were established to document the presence of myocardial ischemia during pacing. All twelve patients had multi-vessel coronary artery disease documented by angiography, with significant narrowing (greater than 75%) of luminal diameter of at least two major vessels. During atrial pacing, they all developed characteristic anginal symptoms, associated with electrocardiographic changes consistent with myocardial ischemia (1 mm flat or downsloping ST segment depression, 0.08 mm in duration in at least 3 consecutive

beats). All patients demonstrated lactate production or a marked decrease from resting lactate extraction, suggesting a shift to anaerobic metabolism.

Ten patients were male and 2 female. Their mean age was 52 years, mean (± SE) resting supine blood pressure 134/86±6/4. Four had a history of myocardial infarction. Five were receiving propranolol. Mean peak pacing rate achieved was 134 beats/min (range 90-150).

Three additional patients, evaluated for a chest pain syndrome, proved to have completely normal coronary arteriographic studies. Atrial pacing in these patients achieved a maximal heart rate equivalent to that in the experimental group (mean 150 beats/min, range 140-160). While these subjects did experience mild chest pain, none demonstrated lactate production or electrocardiographic changes. These patients, therefore, constituted a control population. Two were male and one female. Average age was 47 years, mean resting supine blood pressure 135/80. None had experienced myocardial infarction, and none were receiving propranolol.

Two of the patients with coronary disease (nos 9 and 10) also were considered controls, because blood samples drawn at heart rates prior to the onset of angina or electrocardiographic changes were assayed for prostaglandins.

Pacing Protocol

All patients were premedicated with diazepam (10 mg, po) before being brought to the catheterization laboratory. A no 7 French Zucker pacing catheter was positioned fluoroscopically 2 cm beyond the orifice of the coronary sinus. Position was confirmed by injection of contrast medium (sodium meglumine and diatrizoate). This catheter was used for coronary sinus sampling and atrial pacing. A no 7 NIH angiography catheter was placed in the ascending aorta for sampling and pressure recording connected through a fluid-filled system to a Statham P23DB transducer (Fig 7). The lead V_5 electrocardiograph was monitored throughout and recorded with the pressure on an Electronics for Medicine DR8 multichannel recorder.

Atropine (0.5 mg, iv) was infused in all patients to prevent atrioventricular block. Pacing then was begun at a rate of 10 beats/min greater than control and usually increased in increments of 10 beats/min each successive min. The end-point for termination of pacing was chest pain described by each patient as similar to his usual anginal symptoms. Angiographic study followed thereafter.

Blood samples were drawn simultaneously from coronary sinus and aorta at rest, during peak pacing (angina), and five min after termination of pacing (recovery). Two ml samples were drawn for

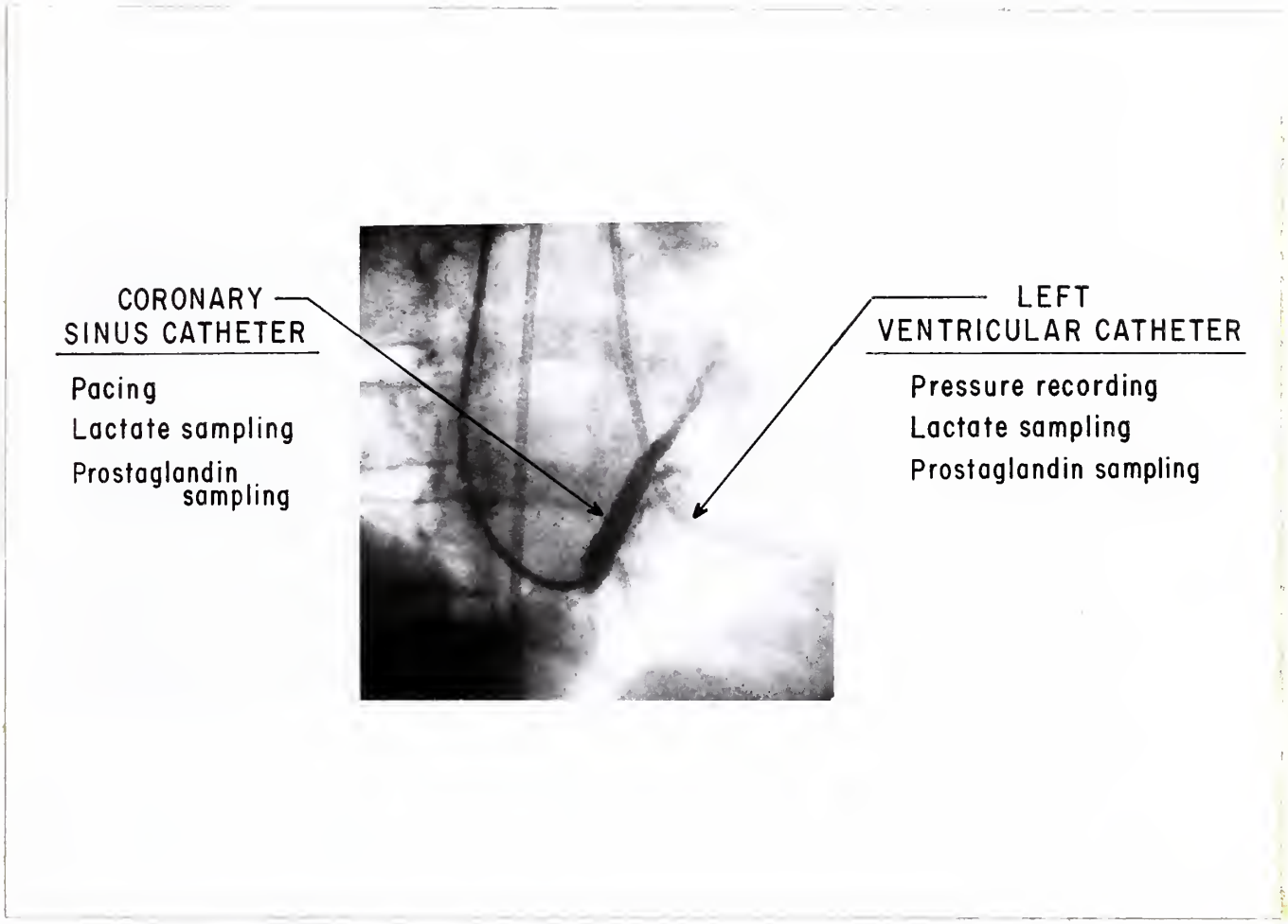

Figure 7. Angiographic study seen in Study III. Contrast medium is injected in the coronary sinus to confirm the position of the catheter. The left ventricular catheter often is pulled back to the aortic arch.

lactate determination and 10 ml samples for prostaglandin assay; the order of sampling for lactate and prostaglandin was randomized. Immediately after withdrawal into plastic syringes, blood samples for prostaglandin analysis were transferred to heparinized tubes which were capped and stored on ice until completion of the catheterization procedure. Lactate samples were immediately transferred into iced perchloric acid for deproteinization and stored on ice.

After deproteinization, samples were centrifuged at 1000 g for 10 min. The plasma was decanted and frozen. Lactate was assayed enzymatically using the Boehringer-Mannheim assay kit; spectrophotometric measurements were made at 366 nm (69). All samples were assayed in duplicate; results are reported as the mean of the values. The inter-assay variation was less than 10%. The data are expressed as lactate uptake (%): $100 \cdot (AO-CS)/AO$, where AO = aortic lactate concentration and CS = coronary sinus lactate concentration. Markedly decreased lactate extraction (positive value) or lactate production (negative value) is considered abnormal and has been correlated with regional myocardial ischemia (70,71) (Fig 4).

Statistical Methods

Prostaglandin levels were expressed as the mean ± SE for aortic and coronary sinus samples. The paired t test was used to analyze differences between aortic and coronary sinus values at rest, during angina, and after recovery. Independent groups were compared by Student's t test. Probability (P) less than 0.05 was taken as a significant difference.

Radioimmunoassay of Prostaglandins

Tubes containing blood for prostaglandin assay were centrifuged at 1000 g for 10 min. The plasma was decanted into scintillation vials which were sealed and stored in a freezer at -20°C. Hemolyzed samples were discarded; extreme care was taken to remove only plasma in the supernatant, leaving behind all formed blood elements including platelets. Assays were completed within two weeks of each pacing study.

Plasma samples were assayed for prostaglandins F, E, and A by radioimmunoassay, as standardized in this laboratory (72). Two ml plasma samples were acidified mildly with 0.1 N hydrochloric acid and extracted twice with 7 ml ethyl acetate. Silicic acid column chromatography was used to separate the three major groups of prostaglandins. Values are expressed as major groups, since the chromatography is unable to differentiate sub-groups (i.e.,

prostaglandins E_1 and E_2). Varying concentrations of methanol in benzene and ethyl acetate were used for elution. Antibodies were generated in rabbits by immunization utilizing prostaglandin bound to bovine serum albumin; the full characterization of the antibodies used in this study has been reported previously (72,73). Charcoal-coated dextran in buffer was used to separate bound and unbound prostaglandin. All assays had at least 65% recovery efficiency. The inter-assay and intra-assay variation was less than 15%. All samples were run in duplicate, but in different sample sized; results are reported as corrected means of the two values. The lower limit of sensitivity of the assay is approximately 0.1 ng/ml.

Study IV: Effect of Indomethacin During Coronary Occlusion In Open-Chest Dogs

Experiments were designed to evaluate the effects of inhibition of prostaglandin synthesis by indomethacin on myocardial ischemia.*

Nineteen healthy dogs, weighing 20-41 kg, were anesthetized with Dial-urethane (Allobarbital, 60 mg/kg; urethane, 240 mg/kg, and monoethylurea, 240 mg/kg) and ventilated mechanically on room air.

* This study was carried out in association with Ralph Kirmser, M.D.

A left thoracotomy was performed in the fourth left intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free approximately 2-3 cm from its origin and isolated with a snare for subsequent occlusion. A short double-lumen catheter was placed in the left atrium, and catheters inserted into the right and left femoral arteries and right femoral vein. All animals received 2000 U heparin iv. The lead II electrocardiogram was monitored continuously. Pressure and electrocardiogram were recorded on a DR-12 Electronics for Medicine multichannel recorder.

The left anterior descending coronary artery was then occluded. At 35 min after occlusion, 10 dogs received indomethacin (10 mg/kg, iv); 9 dogs received only saline, the indomethacin vehicle.

Epicardial ST segment mapping was performed according to the method described by Maroko and co-workers (74). A saline-soaked, cotton wick electrocardiographic probe was applied lightly to approximately 18 sites on the anterior surface of the left ventricle. These sites were distributed in areas supplied by the occluded artery, as well as areas remote from it and presumably adequately perfused. Epicardial electrograms, obtained at a sensitivity of 1 millivolt per mm amplitude, were obtained 5 min prior to occlusion of the left anterior descending coronary artery and every

5 min after occlusion for 80 minutes. ST segment elevation was measured at 100 milliseconds after the onset of the QRS complex. Any site which exhibited a QRS exceeding 65 milliseconds in duration on any single tracing was excluded from analysis for the entire experiment, thus eliminating sites manifesting conduction defects. The average ST segment elevation and the number of sites with ST segment elevation exceeding 2 millivolts were determined at 30, 60 and 75 min.

Regional myocardial blood flow was obtained at 30, 60 and 75 min following coronary artery occlusion using 15 micron carbonized microspheres, labelled with cerium-141, strontium-85, and chromium-51. Immediately before use, the microspheres were thoroughly agitated for 10 min. Approximately 1.5 to 2 million microspheres were injected into the left atrium for each measurement. Arterial reference blood samples were withdrawn from the femoral artery at 15 ml/min for 2 min using a Harvard pump. This also was performed at 30, 60 and 75 min after occlusion.

At 85 min, the heart was excised rapidly. Epicardial fat, vessels, and connective tissue and endocardial connective tissue were dissected away meticulously. Multiple 1 to 2 g biopsies were obtained from the distribution of the left anterior descending coronary artery.

In addition, four biopsies also were taken from the distribution of the left circumflex coronary artery. These biopsies were weighed and counted.

The radioactivity in the myocardial biopsies and in the reference blood samples due to each of the three gamma emitting radionuclides was measured in a well-type sodium iodide scintillation counter using differential spectrometry. Regional myocardial blood flow (ml/100 g/min) was calculated using the following formula: $MBF=(C_B \times RBF/C_R) \times 100$, where MBF equals regional myocardial blood flow, C_B equals counts/g in the biopsy, RBF equals the reference blood flow (15 ml/min), and C_R equals total counts in the reference blood sample. Biopsies with myocardial blood flow less than 30 ml/100 g/min at 30 min were pooled for each dog and defined a zone of severe ischemia. Those biopsies with flows between 30 and 60 mg/100 g/min at 30 min also were pooled for each dog and defined a zone of moderate ischemia. The biopsies from the distribution of the left circumflex coronary artery defined the non-ischemic zone for each dog. Regional myocardial blood flow was also determined in this zone at 30 min.

Statistical Methods

Data from animals treated with indomethacin were compared to those treated with saline at 30, 60, and 75 min after occlusion by unpaired t-test. Because each determination of myocardial blood flow in the moderately and severely ischemic zones was based on a different number of biopsies, the group means were weighted by the number of samples (68).

RESULTS

Study I

All 7 animals demonstrated release of prostaglandin F after occlusion (Table 1). Mean post-occlusion aortic levels were 0.26 $\pm$ 0.1 ng/ml, while coronary sinus were 0.67 $\pm$ 0.1 ng/ml ($p < 0.001$). Three of these animals showed greater than 1 ng/ml prostaglandin F release.

In 6 of 7 experiments, coronary sinus levels of prostaglandin E were significantly greater than aortic after occlusion. Aortic prostaglandin E averaged 0.24 $\pm$ 0.01 ng/ml, while coronary sinus was 0.44 $\pm$ 0.01 ng/ml ($p < 0.001$) after occlusion.

There were no significant post-occlusion differences between aorta and coronary sinus prostaglandin A in any individual animal or for the group of animals ($p > 0.05$).

Before coronary occlusion (baseline), there were no significant arterio-venous differences (Fig 8). After occlusion, maximal release of prostaglandin E averaged 0.76 $\pm$ 0.23 ng/ml. Maximal prostaglandin F averaged 0.91 $\pm$ 0.33 ng/ml.

In the 2 control experiments, there was no detectable release of prostaglandin due to experimental manipulations or blood withdrawal (Table 2).

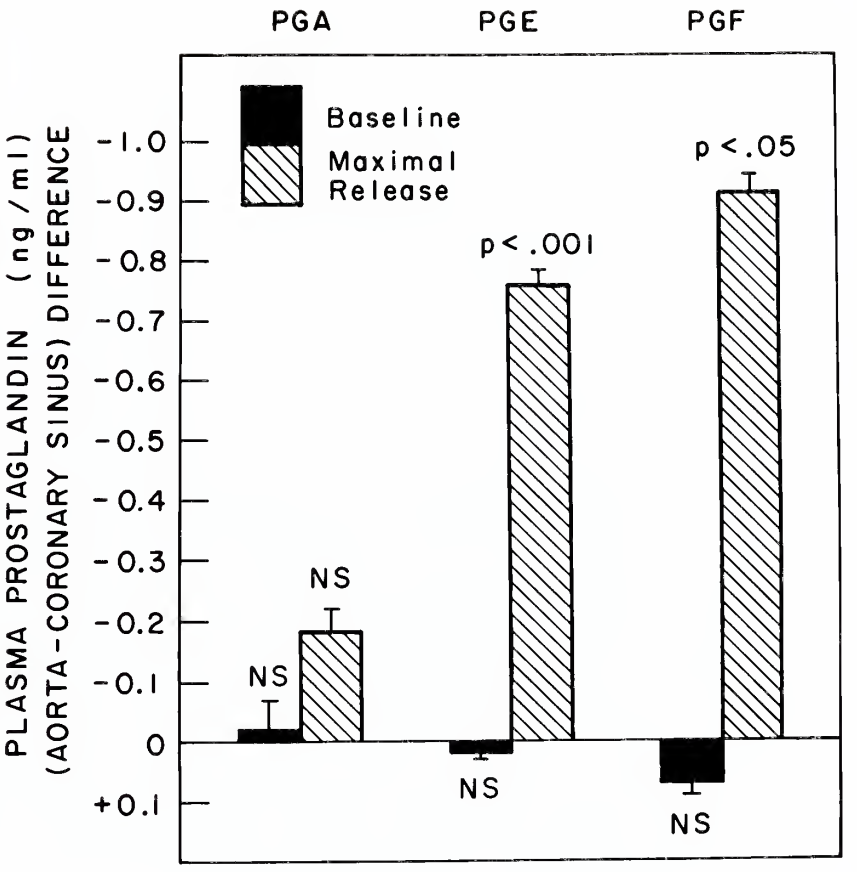

Figure 8. Mean aorta-coronary sinus prostaglandin differences at baseline and at time of maximal prostaglandin release following left anterior descending coronary artery occlusion in 7 dogs. Negative values indicate cardiac prostaglandin release. Vertical bars represent standard errors. Statistical significance of coronary sinus prostaglandin levels compared to aortic levels was determined by a paired t test. NS = Not significant.

Release of prostaglandin E or prostaglandin F occurred within 10 min of occlusion in all animals. Once present, prostaglandin release was maintained until the animal died or until the experiment was terminated. Prostaglandin release was greater in magnitude, and appeared earlier, in those animals surviving less than 20 min.

Study II

Plasma prostaglandin E and prostaglandin F levels, measured for a representative experiment, are shown in Figs 9 and 10. Aortic prostaglandin E remained constant following occlusion, while both coronary sinus (ischemic drainage) and great cardiac vein (non-ischemic drainage) prostaglandin E concentrations were elevated significantly. Aortic prostaglandin F also remained constant throughout the experiment. Proximal coronary sinus drainage, predominantly representing the ischemic region, contained elevated prostaglandin F levels immediately after occlusion and throughout the study. However, there was no release of prostaglandin F from the normal zone drained by the distal great cardiac vein. There was no prostaglandin A release from either site.

Five of 6 animals demonstrated significant release of prostaglandin E from the great cardiac vein drainage, and all 6 showed

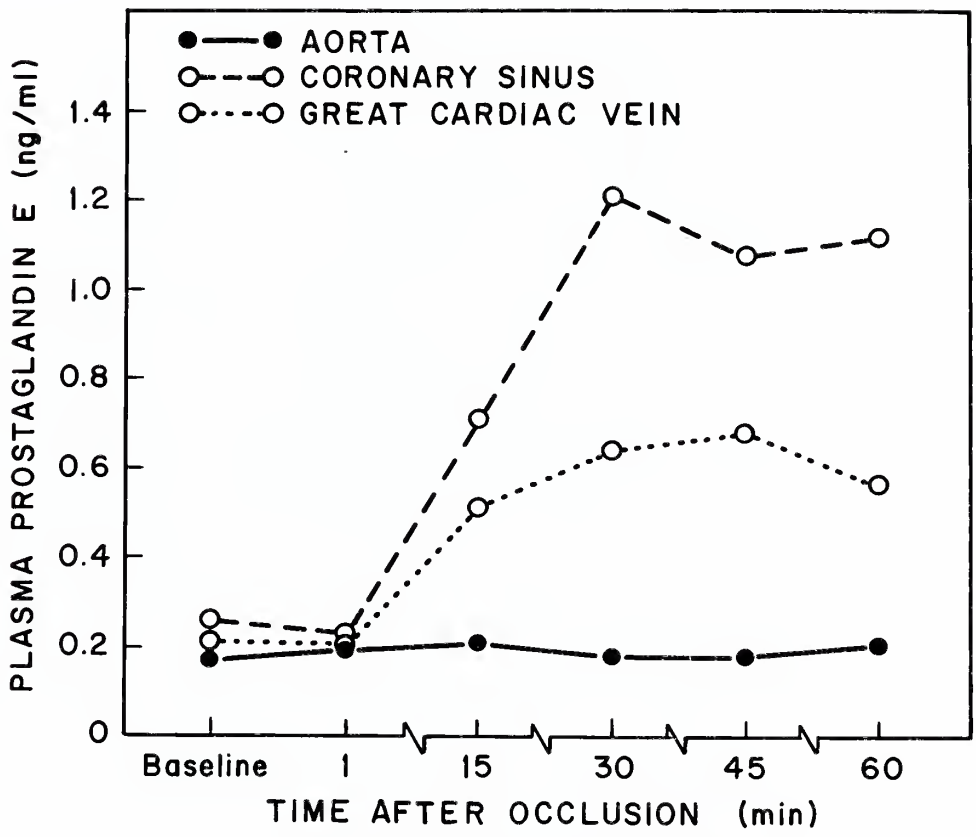

Figure 9. Prostaglandin E levels before and after left circumflex coronary artery occlusion in a representative dog (II). The animal died from ventricular arrhythmias about 65 minutes after occlusion.

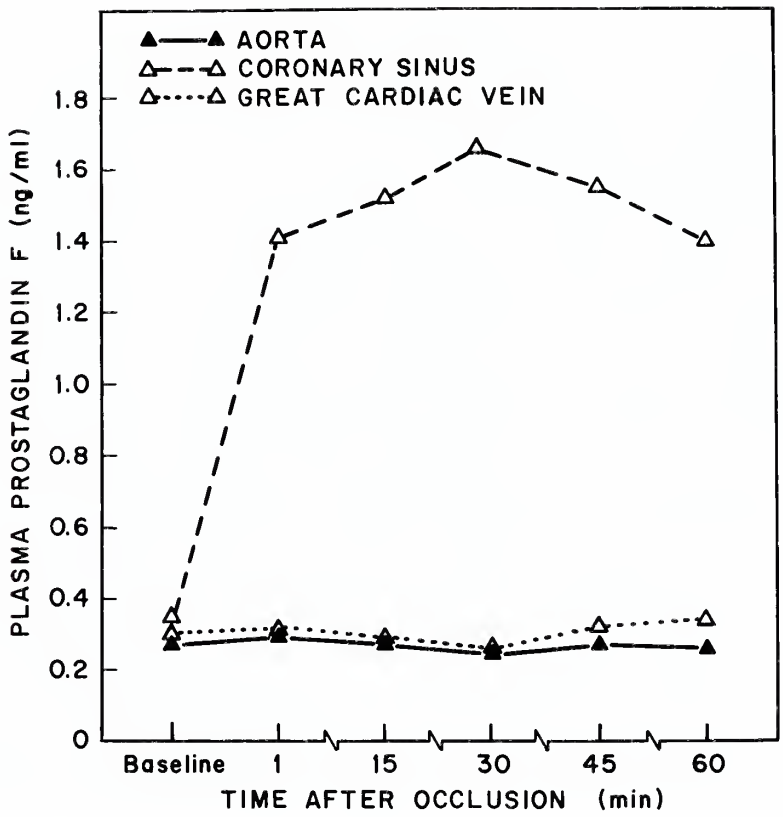

Figure 10. Prostaglandin F levels before and after left circumflex coronary artery occlusion in the same dog as in Figure 9.

release from the coronary sinus (Table 3). Mean aortic prostaglandin E was 0.21 $\pm$ 0.01 ng/ml. Mean great cardiac vein was 0.55 $\pm$ 0.02 ng/ml ($p < 0.001$), and mean coronary sinus prostaglandin E, 1.07 $\pm$ 0.04 ng/ml ($p < 0.001$, compared with aortic levels).

All 6 animals demonstrated release of prostaglandin F from the ischemic region (proximal coronary sinus), while only one animal showed release from the non-ischemic region (distal great cardiac vein). Mean aortic prostaglandin F was 0.32 $\pm$ 0.01 ng/ml ($p < 0.05$). Coronary sinus prostaglandin F was significantly elevated at 1.69 $\pm$ 0.03 ng/ml ($p < 0.001$).

There was no significant release of prostaglandin A in any of the individual animals following coronary occlusion. However, there was a small, but statistically significant increase in mean great cardiac vein prostaglandin A. Relative to the sensitivity of the assay, this difference (0.03 ng/ml) is too small to be meaningful.

When arterio-venous differences for prostaglandin were analyzed, no significant difference was noted prior to occlusion. The aorto-venous differences at the time of maximal prostaglandin release post-occlusion are shown in Fig 11. Prostaglandin F was released only from the ischemic zone, with a maximal aorto-venous

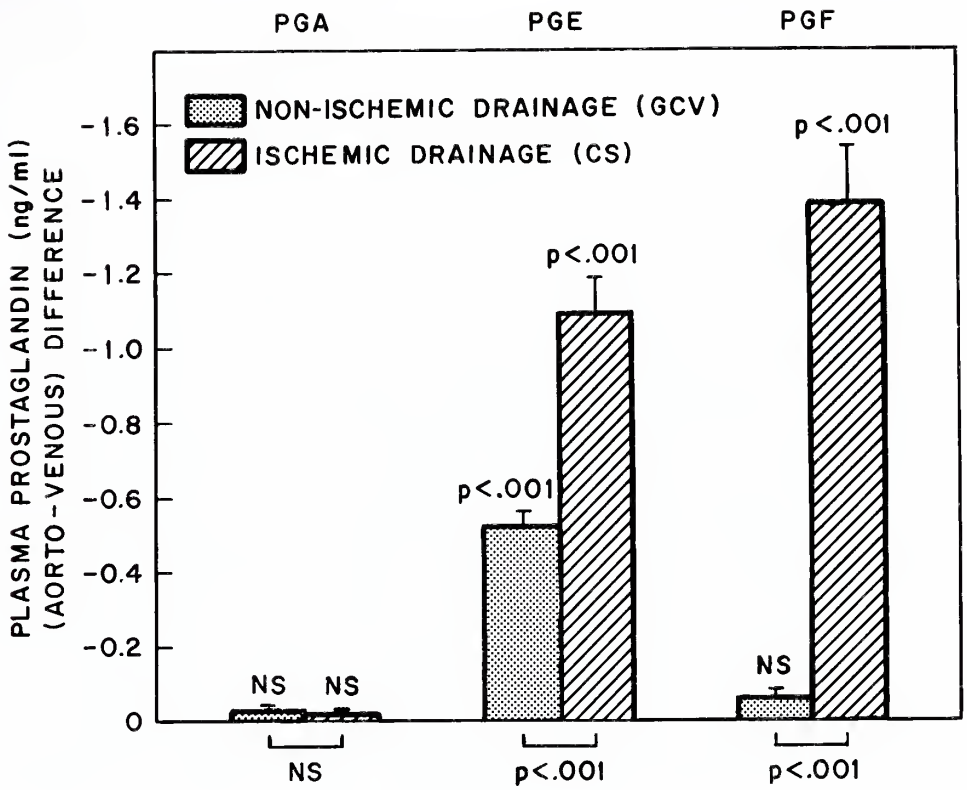

Figure 11. Mean aorto-venous prostaglandin differences at time of maximal prostaglandin release following left circumflex coronary artery occlusion in 6 dogs (II). Negative values indicate cardiac prostaglandin release. Vertical bars represent standard errors. Statistical significance of coronary sinus (CS) or great cardiac vein (GCV) prostaglandin levels compared to aortic levels was determined by a paired t test.
The venous sampling sites were similarly compared.
NS = Not significant.

difference of -1.39 $\pm$ 0.15 ng/ml. In contrast, prostaglandin E was released from both ischemic and non-ischemic regions, -1.09 $\pm$ 0.09 and -0.52 $\pm$ 0.04 ng/ml, respectively.

Study III

At rest, prior to pacing, there was no difference discernible between aortic and coronary sinus prostaglandin F levels ($p > 0.05$). During angina, ST segment changes, and lactate production (or markedly decreased extraction) suggesting myocardial ischemia, there was significant release of prostaglandin F. A myocardial aorto-venous difference of -0.30 $\pm$ 0.04 ng/ml ($p < 0.001$) was found. At recovery, coronary sinus prostaglandin F was still elevated above aortic levels. Throughout the pacing study, aortic levels were constant (Fig 12).

The release of prostaglandin F occurred in 11 of 12 patients (Fig 13). The response of the 5 patients receiving propranolol was indistinguishable from that of the patients who did not receive propranolol ($p > 0.05$). The one patient (no 4) who did not demonstrate prostaglandin F release was indistinguishable from the other patients by clinical or angiographic criteria. None of the 3 control patients with normal coronary anatomy manifested prostaglandin release.

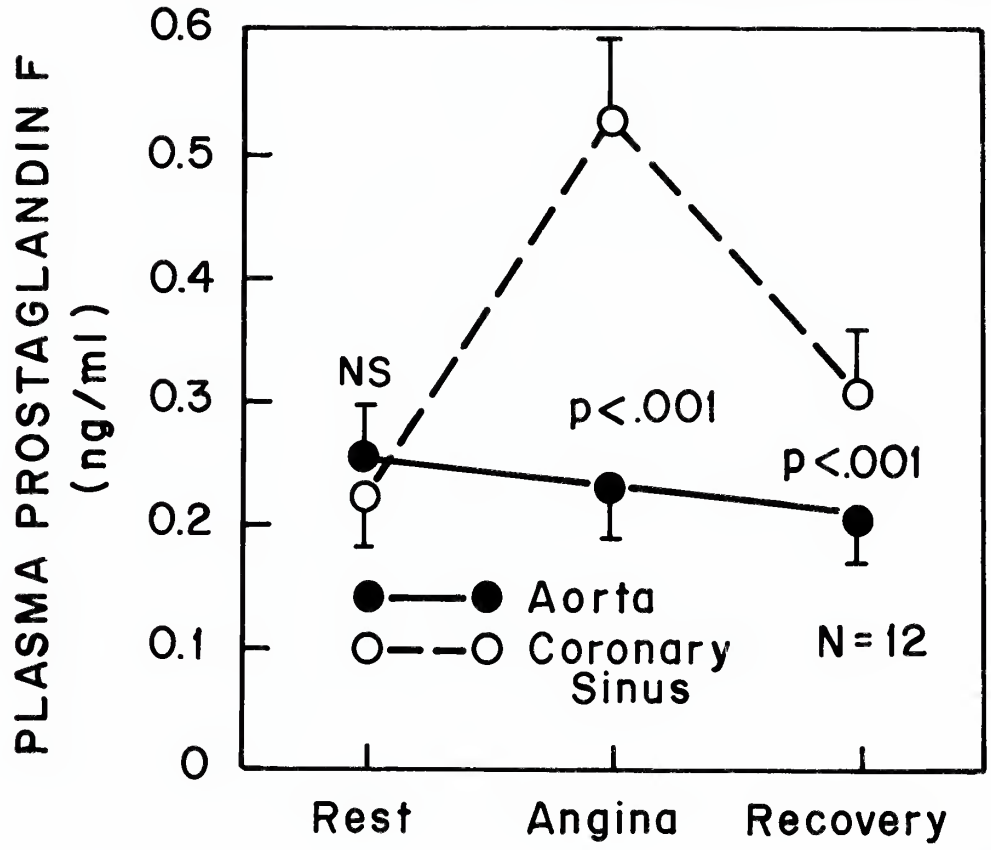

Figure 12. Plasma prostaglandin F levels in 12 patients with coronary artery disease. "Recovery" was 5 minutes after conclusion of pacing. Statistical significance was determined by a paired t test. NS = Not significant.

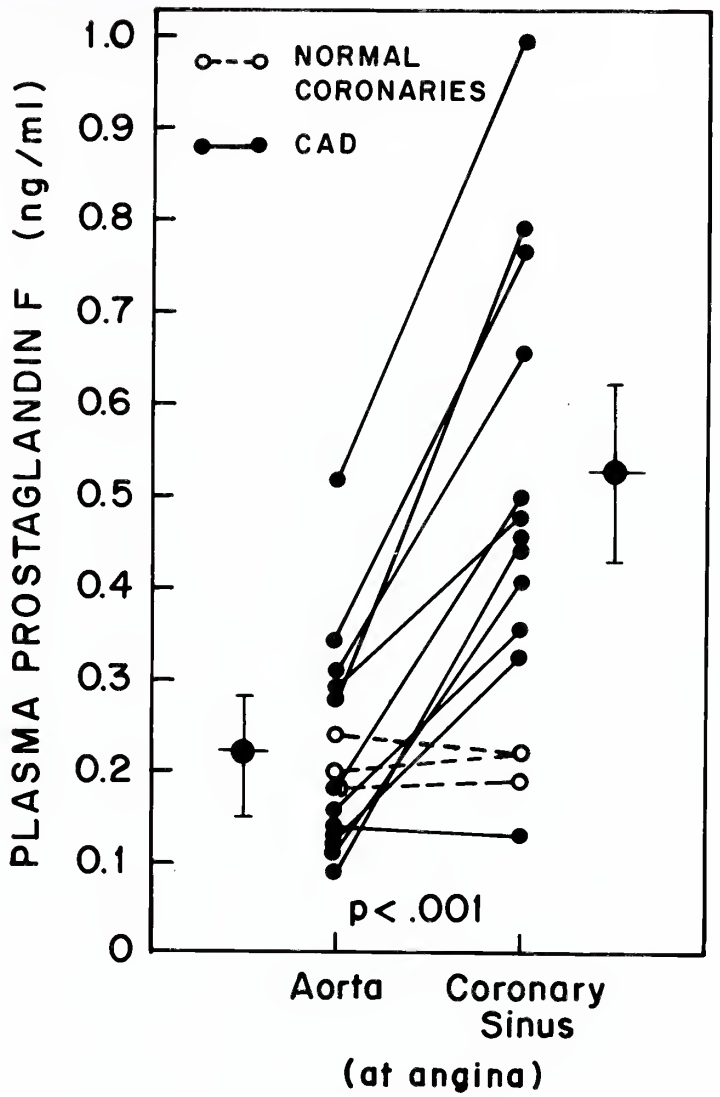

Figure 13. Plasma prostaglandin F levels at peak pacing rate achieved in 12 patients with coronary artery disease and in 3 patients with normal coronaries. All patients in the first group had typical anginal pain, electrocardiographic changes consistent with ischemia, and myocardial lactate production at peak pacing rate, while control patients only had mild chest pain. Mean prostaglandin F for the group of 12 patients is shown by the circled bar on either side of the panels; vertical bars represent standard errors. Statistical significance refers to paired comparison of aortic and coronary sinus prostglandin F levels in the group of 12 patients. CAD=Coronary artery disease.

In the study group, the mean lactate uptake was -10.2%, indicative of myocardial lactate production, while in the control group, mean lactate uptake was +25.4%. Although there is an association between lactate production (or markedly decreased extraction) and myocardial prostaglandin F release in patients with multivessel coronary artery disease, a quantitative correlation between these 2 biochemical parameters could not be established (Table 4). At angina, 7 of the patients with coronary disease had profound production of lactate, ranging from -10% to -26% uptake. Five had either moderate production or diminished extraction. The mean prostaglandin F release in these 2 groups was not different ($p < 0.05$). In addition, there was no relationship between the magnitude of prostaglandin F release and the extent or distribution of coronary arterial lesions, the depth of ST segment depression, or the maximal heart rate achieved on pacing.

Two patients were studied at heart rates preceding the development of angina (Fig 14). Aortic prostaglandin F levels were constant at all pacing rates. Neither patient demonstrated prostaglandin F release at rest. Patient 9 had no release at rates of 70 or 80 beats/min, but did manifest prostaglandin F release when angina and ST segment depression developed (heart rate 90). Similarly, patient 10 showed prostaglandin F release only at a rate producing

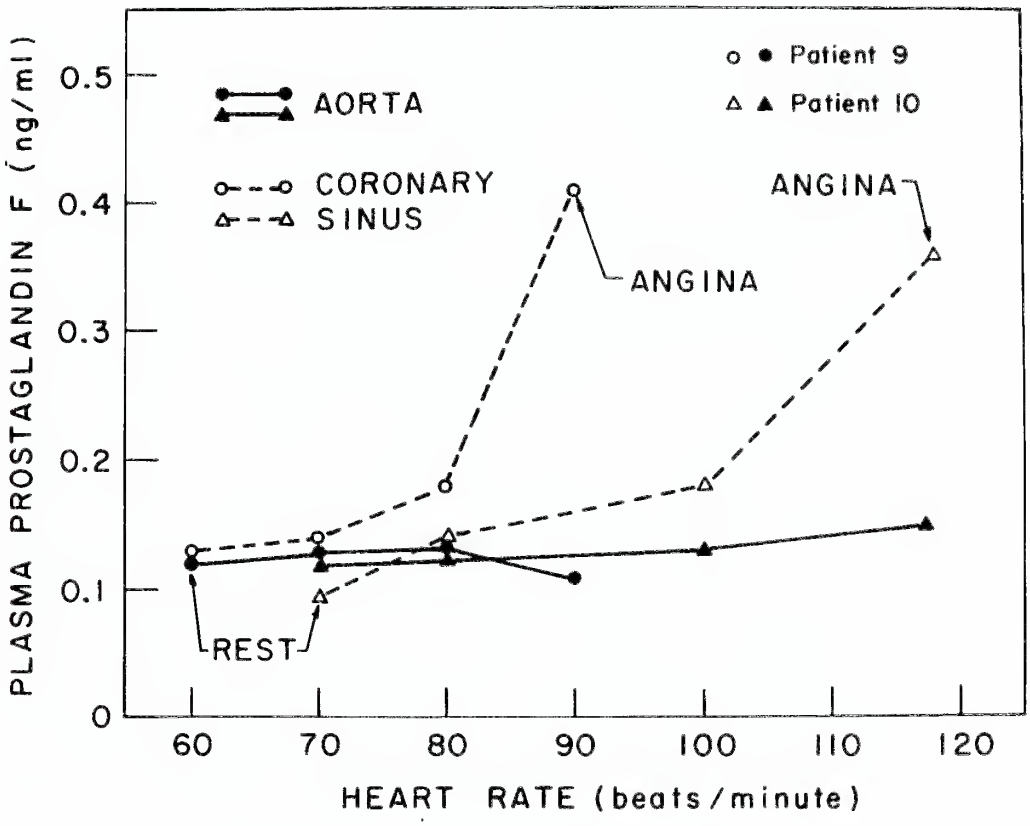

Figure 14. Plasma prostaglandin F levels at rest and during atrial pacing in 2 patients. There was no cardiac release of prostaglandin F at sub-anginal heart rates.

angina (120 beats/min), but not at 70, 80 or 100 beats/min.

Prostaglandin E release was not observed at rest or recovery. At the time of angina, there was a small, but statistically significant aorto-venous difference. Although this -0.10 $\pm$ 0.03 ng/ml difference is statistically significant, it is at the lower limit of sensitivity of the radioimmunoassay technique used (0.10 ng/ml), and therefore cannot be the basis for definitive conclusions. The trend shown (Fig 15) is similar to that for prostaglandin F, yet only 6 of the 12 patients had aorto-coronary sinus differences greater than -0.10 ng/ml. Prostaglandin A release was not observed at rest, during angina, or after recovery (Fig 16).

Study IV

The experiments in 9 control animals demonstrated an immediate increase in ST segment elevation following occlusion. Both the average ST segment elevation and the number of sites with greater than 2 mm elevation rose progressively during the first 30 min, but remained stable from 30 min through the end of the observation period. Myocardial blood flow measurements in control animals were unchanged at 30, 60, and 75 min in each of the 3 zones.

Indomethacin did not affect heart rate, mean arterial blood pressure,

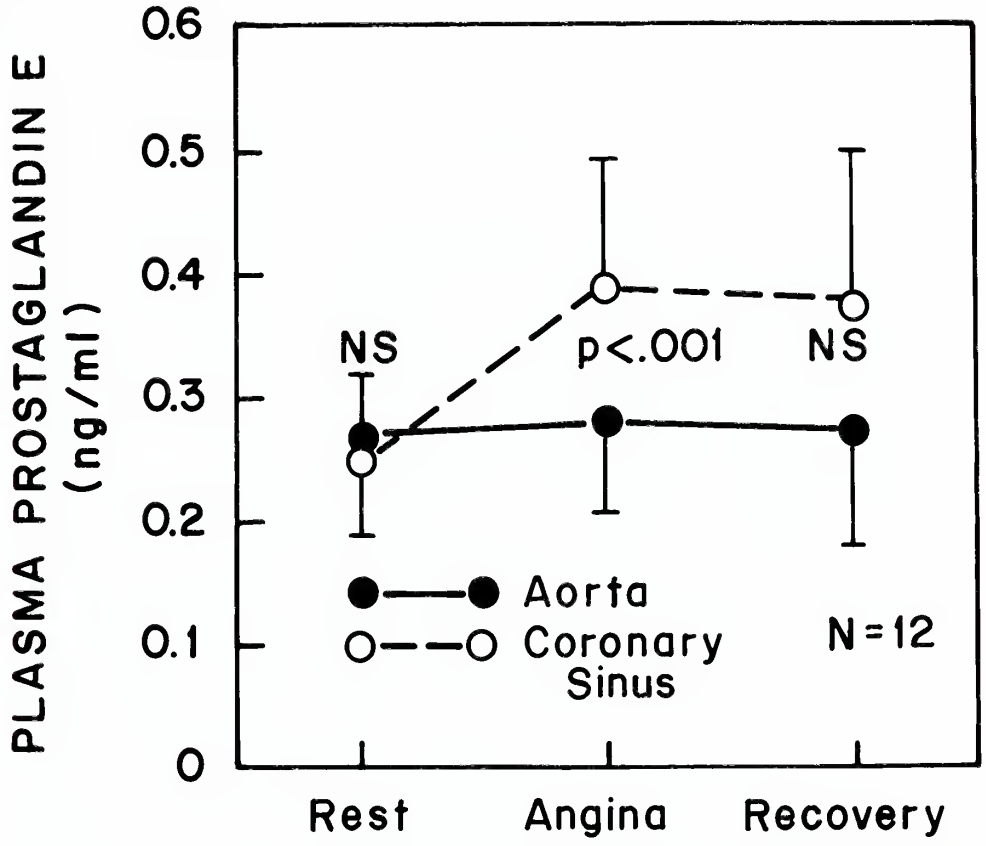

Figure 15. Plasma prostaglandin E levels in 12 patients with coronary artery disease. Although statistically significant, the aorta-coronary sinus difference at angina is too small an absolute difference on which to base conclusions (See text). NS = Not significant.

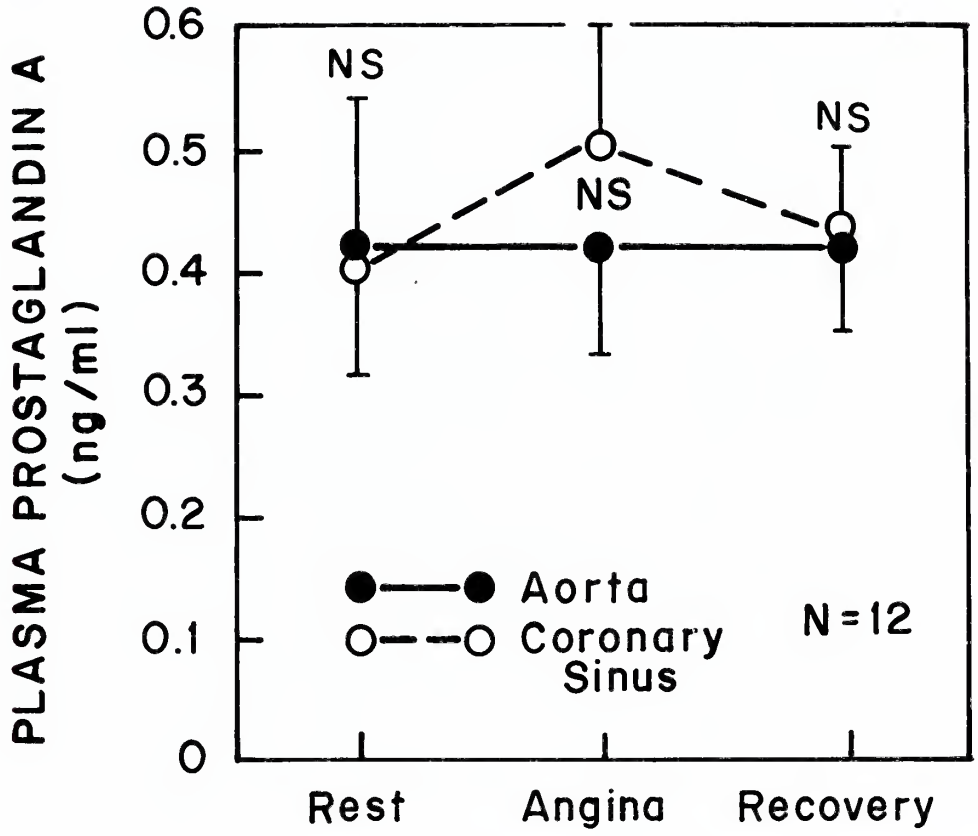

Figure 16. Plasma prostaglandin A levels in 12 patients with coronary artery disease. There was no release of prostaglandin A at any time. NS = Not significant.

or their product. At 60 min, the double product averaged 22.6 ± 0.5 mm Hg x beats x min^{-1} x 10^3 for the control group and 22.1 ± 0.4 for the indomethacin group ($p > 0.05$). Myocardial blood flow in the normal zone was not altered by indomethacin (control, 126 ± 12 ml/100 g/min; indomethacin, 122 ± 14; at 60 min, $p > 0.05$). These data suggest that myocardial oxygen demand remained constant during the course of each study.

However, in comparison to control animals, those treated with indomethacin manifested a significant increase in both the average ST segment elevation (Fig 17) and the number of epicardial sites with at least 2 mm elevation (Fig 18). At 60 min after occlusion, which is 25 min after indomethacin administration, average ST segment elevation was 4.9 ± 1.1 mm (compared to control, 1.4 ± 0.6; $p < 0.025$) and number of sites was 10.3 ± 1.1 (compared to control, 3.9 ± 1.2; $p < 0.001$). Furthermore, myocardial blood flow in the moderately and severely ischemic zones was diminished at 60 and 75 min in the animals given indomethacin ($p < 0.05$) (Figs 19,20).

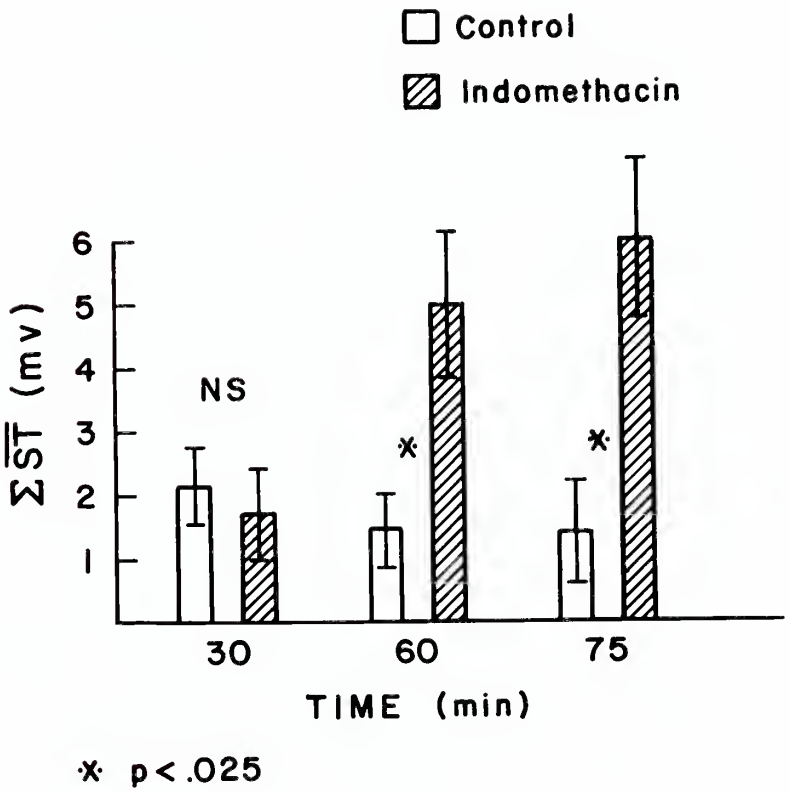

Figure 17. Average ST segment elevation ($\Sigma\overline{ST}$) in 10 dogs given indomethacin and 9 given saline (control). Indomethacin, administered 35 minutes (min) after occlusion, caused a significant increase in $\Sigma\overline{ST}$ at 60 and 75 min. Vertical bars represent standard errors. Statistical significance was determined by unpaired t test. NS = Not significant.

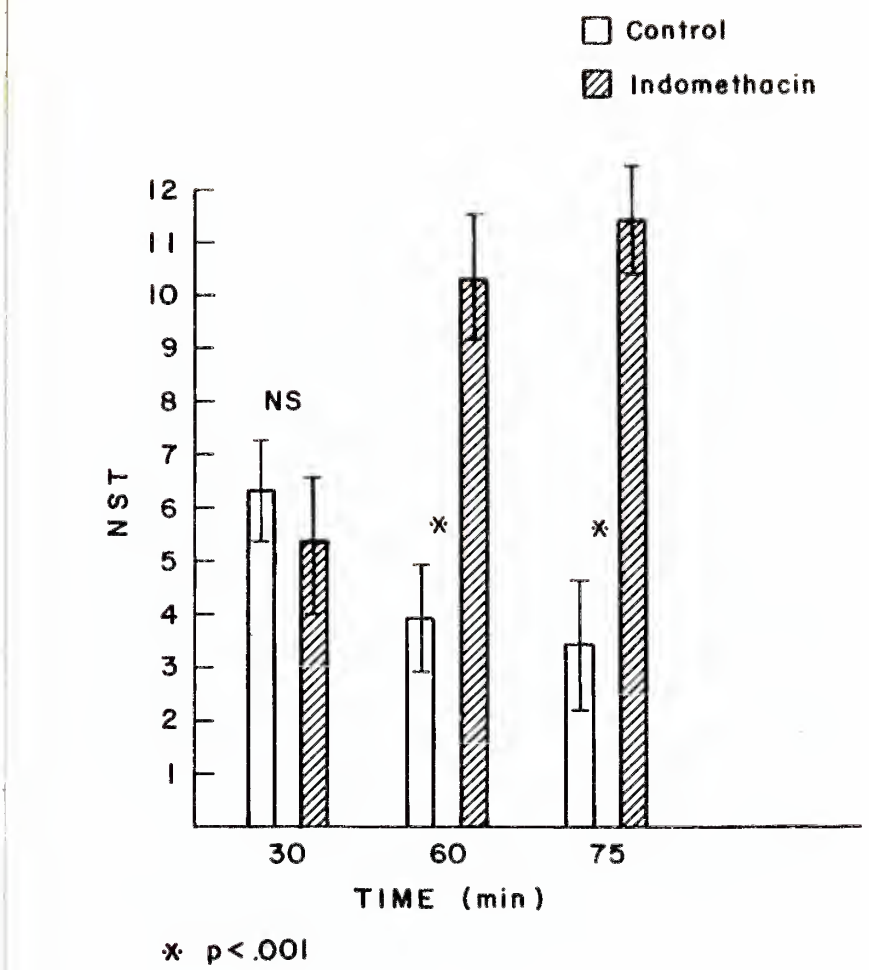

Figure 18. Number of epicardial sites with ST segment greater than 2 mm (NST) in 10 dogs given indomethacin and 9 given saline (Control). Indomethacin caused a significant increase in NST at 60 and 75 minutes (min) after occlusion. NS = Not significant.

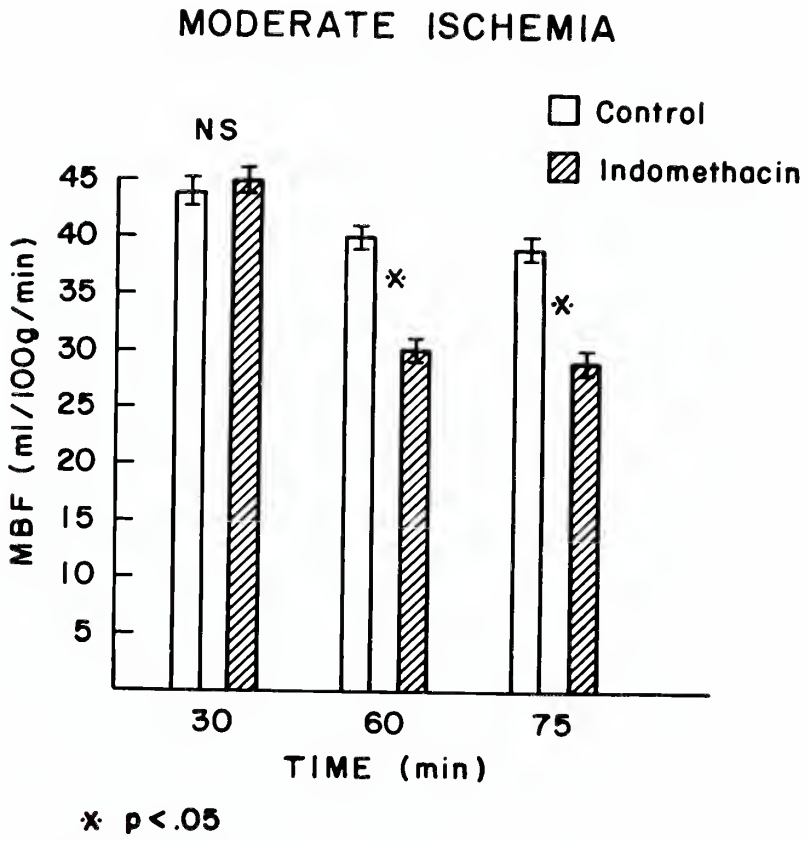

Figure 19. Myocardial blood flow (MBF) in the zone of moderate ischemia. In the 10 dogs given indomethacin, MBF was significantly less at 60 and 75 minutes (min) after occlusion than in the 9 given saline (control). Statistical significance was determined by unpaired t test. NS = Not significant.

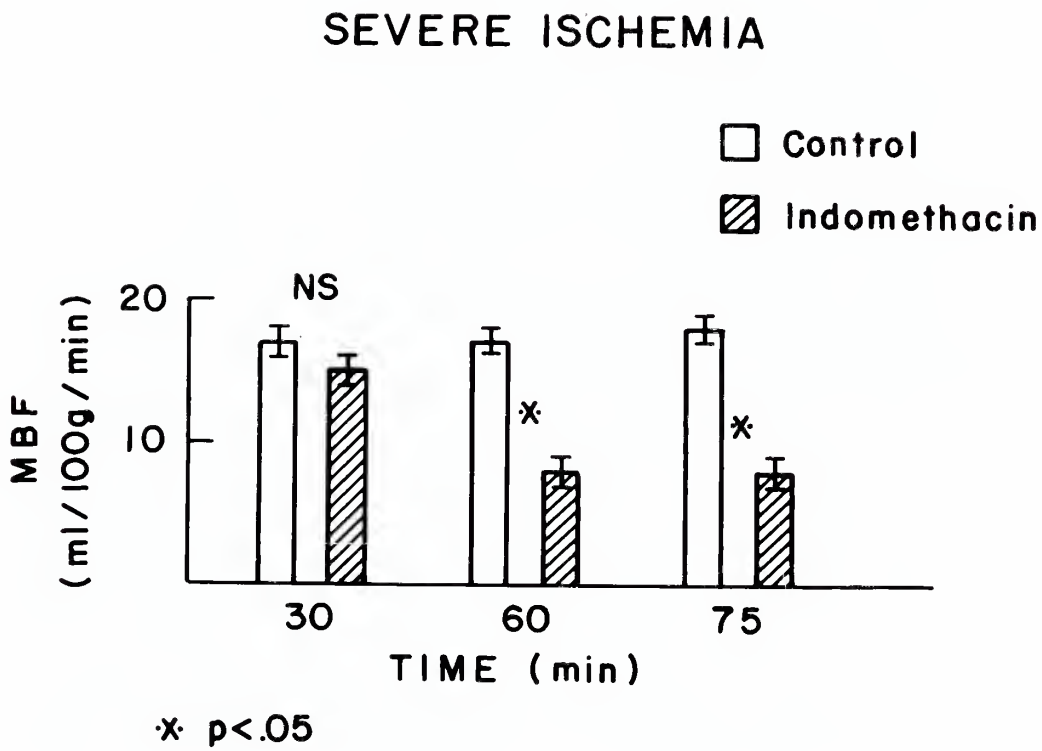

Figure 20. Myocardial blood flow (MBF) in the zone of severe ischemia. In the 10 dogs given indomethacin, MBF was significantly less at 60 and 75 minutes (min) after occlusion than in 9 given saline (control). NS = Not significant.

DISCUSSION

Studies I and II demonstrate the release of prostaglandin E and prostaglandin F from the canine heart during myocardial ischemia. This finding in the intact anesthetized dog is in agreement with earlier studies using the perfused rabbit heart (40-45) and the work by Alexander et al (46) using the canine heart-lung preparation. In contrast to the data of Kraemer et al (47), release of prostaglandin A was not shown at any time. The validity of prostaglandin A values in the dog may be questioned because of the presence in canine plasma of a prostaglandin A isomerase, which converts prostaglandin A to prostaglandin B (75).

The release of both prostaglandin E and prostaglandin F from the heart during ischemia resembles the findings of McGiff et al (36) in the kidney. During canine renal ischemia, prostaglandin E and prostaglandin F were identified in renal vein blood by bioassay and thin layer chromatography. Recent studies from that group (76) have demonstrated the enzymatic conversion of prostaglandin E to prostaglandin F in the rabbit kidney, suggesting that some of the prostaglandin F measured may have been released as prostaglandin E. Similar conversion might also occur in the heart.

Prostaglandin E was found in the venous effluent from both

ischemic and non-ischemic regions, while prostaglandin F release was limited to the ischemic region. These findings suggest that the roles of prostaglandin E and prostaglandin F may be different. Several earlier studies have demonstrated different physiologic effects for these two prostaglandins in the cardiovascular system. Prostaglandin E (but not prostaglandin F) increases myocardial adenyl cyclase (14), increases vascular Na^+ - K^+ - ATPase (77), and inhibits adrenergic transmission by post-junctional depression (29). On the other hand, prostaglandin F in low doses (but not prostaglandin E) acts at the cardioregulatory and vasomotor centers of the hind brain to regulate vagal tone to the heart (78).

The release of prostaglandin F from the human heart during myocardial ischemia was demonstrated in study III. The usefulness of this finding as a diagnostic test for coronary artery disease cannot be defined from this investigation. These patients were carefully chosen for inclusion in this protocol, all having transient myocardial ischemia documented by their typical anginal symptoms, lactate abnormality, and electrocardiographic changes. The release of prostaglandin F does appear to be a specific event occurring during myocardial ischemia. It was not shown at heart rates unaccompanied by chest pain or significant ST segment depression in two of these patients, nor in control patients with angiographically

normal coronary arteries subjected to the same pacing protocol.

All patients in this study received diazepam and atropine, and 5 also were receiving propranolol. Although these agents conceivably could affect prostaglandin metabolism, neither propranolol nor atropine have been found to alter the hemodynamic response to prostaglandins (21,23). Furthermore, the lack of prostaglandin release in the normal control patients casts doubt on any direct effect of diazepam or atropine on prostaglandin release.

The levels of prostaglandins found in this study are comparable, although not identical, to those previously reported for radioimmunoassay (79). Others have presented venous concentrations, which are not necessarily interchangeable with either coronary sinus or aortic levels. In general, the radioimmunoassay technique yields prostaglandin levels higher than those observed using mass spectroscopy (54). This has been noted frequently, but the reasons for the difference are not readily apparent at this time.

In the present investigation, prostaglandin F release was the dominant finding during ischemia. However, the previous studies (I and II) demonstrated comparable release of prostaglandins E and F during acute coronary occlusion in the closed-chest dog. The fact that prostaglandin F release during ischemia in man was more pronounced than prostaglandin E release may reflect a species

difference relative to the animal study or to the more severe impact of acute coronary occlusion in the experimental preparation.

The lack of a correlation between prostaglandin F release and lactate production is not surprising. Lactate is supplied to the myocardium as a substrate in arterial blood, extracted by normal myocardium, and produced by the myocardium in the presence of ischemia and anaerobic metabolism. The net result is an algebraic summation producing the aorto-venous difference (69). The kinetics of lactate uptake and release are modified by coronary blood flow, which was not measured in this study. Lactate production or decreased extraction, however, have been considered reliable indicators of regional myocardial ischemia, especially if considered together with ST segment depression and anginal pain (71). Prostaglandin F, on the other hand, is present only in minimal amounts in arterial blood and probably does not serve a systemic role.

The significance of the demonstrated release of prostaglandins from the heart was examined in the experiments (IV) with indomethacin, a potent inhibitor of prostaglandin synthetase (Fig 2). Indomethacin treatment during coronary occlusion resulted in immediate deleterious effects. Epicardial ST segment elevation, an indicator of myocardial ischemia increased, and regional

myocardial blood flow decreased. These directional changes suggest that endogenous cardiac prostaglandins play a beneficial role during myocardial ischemia.

The dose of indomethacin used has been shown to completely block prostaglandin synthesis in all tissues studied (37-39). However, nonspecific actions of indomethacin on the heart (15), independent of its effects on prostaglandin biosynthesis, cannot be excluded from interpretation of these results. Similar studies using other inhibitors (Fig 2) are presently underway. In addition, confirmation of indomethacin's inhibition of cardiac prostaglandin release is being performed in this laboratory.

There are some potential problems in the use of epicardial ST segment mapping to quantify myocardial ischemia. The electrophysiologic basis of ST segment elevation is poorly understood; it appears to depend upon variables, such as arterial oxygen tension, ventricular conduction, sympathetic stimulation, and multiple vectors determined by the distribution of ischemia (80,81). A linear correlation between ST segment changes and regional myocardial blood flow has not been established (82). Nevertheless, a good relationship has been found between ST segment elevation and subsequent evolution of myocardial necrosis (83).

The principal defect of the microsphere technique for measuring

regional myocardial blood flow is non-randomicity of distribution of the microspheres, leading to variable measurements even in normal myocardium. Estimates of the error of this technique have ranged from 10 to 14% (84).

Errors are maximal in the zones of severe ischemia, since fewer microspheres reach these zones, leading to poorer statistics for isotope counting. However, the number of microspheres utilized for this study is at the upper range of those shown to be free of effects on myocardial contractility. Thus, even in poorly perfused zones of myocardium, microsphere delivery should be adequate.

The balance between oxygen demand and oxygen supply is depicted in Fig 21. When demand exceeds supply and this equality no longer exists, ischemia and its manifestations result (85). The major determinants of oxygen consumption are the contractile state of the heart, the heart rate, and the intraventricular wall tension, which is dependent upon left ventricular volume and arterial blood pressure. In terms of oxygen supply, aortic perfusion pressure and coronary vascular resistance determine coronary blood flow. The transmural distribution of flow (endocardial and epicardial), as well as total flow, are of importance in providing oxygen to the myocardium, especially because the subendocardial layers of the wall are most susceptible to ischemia. These factors, combined

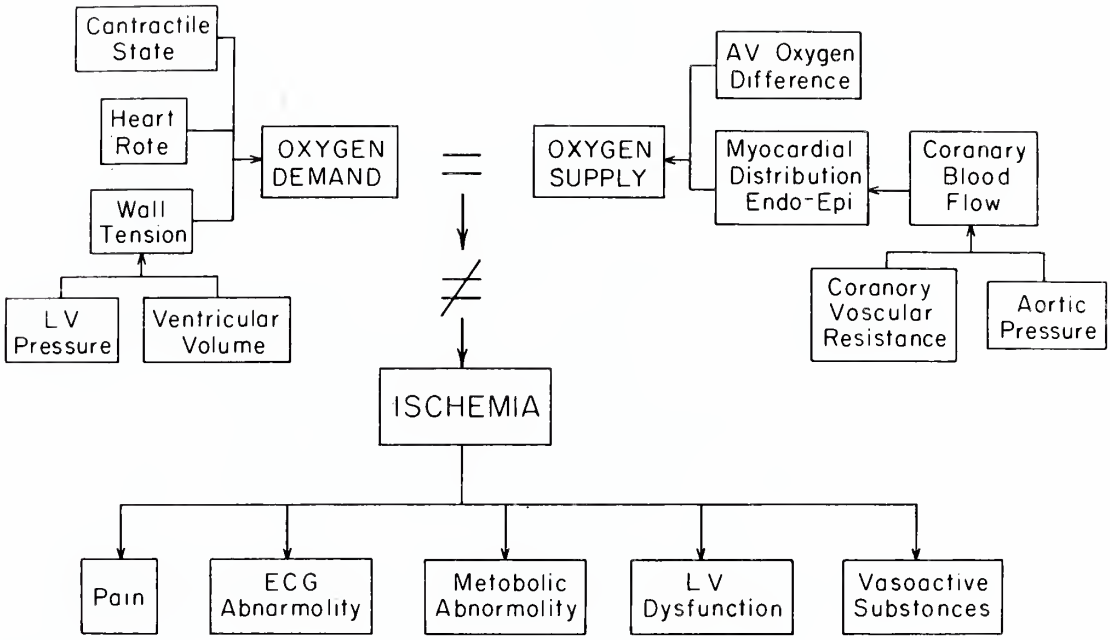

Figure 21. Systems diagram showing the balance between myocardial oxygen supply and demand. The consequences of ischemia are shown below. Release of vasoactive substances may lead to a negative feed back loop primarily affecting coronary vascular resistance. (From Ross, reference 65).

with the oxygen-carrying capacity of the blood, ultimately determine oxygen supply.

In the normal circulation, coronary blood flow varies to meet myocardial oxygen demands. If local coronary flow fails to adjust to increased oxygen requirements, ischemia results (86). The processes by which local coronary flow is regulated have been studied by examining the coronary sinus effluent for substances which may modulate the coronary vascular response to myocardial ischemia. Lactate (70,71) and potassium (87) have been shown to be released from the myocardium during ischemia. The vasoactive compound, adenosine (88), and components of the kallikrein system (bradykinin) (89) also have been identified in coronary venous blood in man.

Referring to Fig 21, there are several important possible feed back loops. For example, vasoactive compounds, such as those mentioned above or prostaglandins, may act to reduce coronary vascular resistance after release and thus diminish ischemia; this represents a beneficial negative loop. In contrast, left ventricular dysfunction may initiate a positive loop that would make the system more unstable. As cardiac performance decreases, ventricular volume, wall tension, and oxygen demands all increase; aortic pressure falls concomitantly, resulting in decreased coronary blood flow.

The association between cardiac prostaglandin release and regional myocardial ischemia in animals and man suggests that this pharmacologically potent substance may also play a physiologic role in the cardiac response to ischemia. However, it should be recognized that the presence of prostaglandin release in this pathophysiologic circumstance does not confirm a causal relationship. Prostaglandins may arise from myocardial cells as a direct response to ischemia or from coronary vascular smooth muscle cells as they become involved in compensatory vasodilitation. In contrast, these compounds could initiate a compensatory hemodynamic or metabolic response after release. Definition of the precise mechanisms involved will require further study.

SUMMARY

The physiologic role of cardiac prostaglandin release during myocardial ischemia was evaluated in four studies.

In 7 closed chest dogs, aortic and coronary sinus blood samples were obtained before, and at intervals after, balloon occlusion of the left anterior descending coronary artery (I). Samples were assayed for prostaglandins F, E and A by radioimmunoassay. All 7 animals demonstrated prostaglandin F release. Mean $\pm$ SE post-occlusion aortic prostaglandin levels were 0.26 $\pm$ 0.1 ng/ml, while coronary sinus levels averaged 0.67 $\pm$ 0.1 ng/ml ($p < 0.001$). In 6 of 7 animals, prostaglandin E also was released. Mean post-occlusion aortic levels were 0.24 $\pm$ 0.01 ng/ml, coronary sinus 0.44 $\pm$ 0.01 ng/ml ($p < 0.001$). There was no release of prostaglandin A. Release of prostaglandins E and F occurred within 10 min of occlusion in all animals and persisted until the animal died.

To examine the site of prostaglandin release, simultaneous samples from aorta, coronary sinus, and great cardiac vein were obtained before and after balloon occlusion of the left circumflex coronary artery in 6 additional studies (II). During left circumflex occlusion, the great cardiac vein drains effluent from normal (non-ischemic) myocardium, whereas coronary sinus drainage

includes blood from both ischemic and non-ischemic zones.

All 6 animals demonstrated release of prostaglandin F from the ischemic region. Mean post-occlusion aortic prostaglandin F was 0.32 $\pm$ 0.01 ng/ml. Coronary sinus prostaglandin F was significantly elevated at 1.69 $\pm$ 0.03 ng/ml ($p < 0.001$), while the great cardiac vein level remained at 0.34 $\pm$ 0.01 ng/ml ($p > 0.05$). Prostaglandin E was released from both ischemic and non-ischemic regions. Mean aortic prostaglandin E was 0.21 $\pm$ 0.01 ng/ml, great cardiac vein 0.55 $\pm$ 0.02 ng/ml ($p < 0.001$), and coronary sinus 1.07 $\pm$ 0.04 ng/ml ($p < 0.001$).

The relationship between myocardial prostaglandin release and myocardial ischemia was studied in 12 selected patients with multivessel coronary artery disease (III). These 12 were chosen for analysis because they developed angina, ischemic electrocardiographic changes, and decreased myocardial lactate uptake during atrial pacing. Simultaneous aortic and coronary sinus blood samples were obtained at rest, during angina, and after recovery and were assayed for prostaglandins F, E and A by radioimmunoassay. Cardiac release of prostaglandin F was observed during angina in 11 of 12 patients. Aortic prostaglandin levels remained constant throughout each study. During angina, the mean $\pm$ SE aorto-venous difference for prostaglandin F was -0.30 $\pm$

0.04 ng/ml ($p < 0.001$); for prostaglandin E, -0.10 ± 0.03 ng/ml ($p < 0.001$). There was no significant release of prostaglandin A. Samples were also drawn at sub-anginal heart rates in two patients. Prostaglandin F was release only during angina. In three control patients with a chest pain syndrome and normal coronary arteries, comparable atrial pacing produced no release of prostaglandins F, E or A.

Epicardial ST segment maps and regional myocardial blood flow, determined by 15 micron microspheres, were measured in 19 open-chest dogs 30, 60 and 75 min after left anterior descending coronary occlusion (IV). Biopsies from the region of the left anterior descending coronary artery with myocardial blood flow less than 30 ml/100g/min at 30 min defined the zone of severe ischemia; biopsies with myocardial blood flow between 30 and 60 defined the zone of moderate ischemia. Biopsies from the circumflex region defined the normal zone. At 35 min, 10 dogs received indomethacin (10 mg/kg, iv) and 9 controls received saline, the indomethacin vehicle.

Indomethacin did not affect heart rate, mean arterial blood pressure, or myocardial blood flow in the normal zone. In contrast, Indomethacin decreased myocardial blood flow in both the severely and moderately ischemic zones ($p < 0.05$). This was associated with a marked increase in the number of epicardial sites with ST segment

elevation greater than 2 mm ($p < 0.001$) and the mean ST segment elevation ($p < 0.025$).

These results, together with the known physiologic and pharmacologic actions of prostaglandins E and F, suggest that their local availability to different myocardial regions is of importance in the cardiac response to ischemia.

Table 1. Mean Prostaglandin levels following coronary occlusion.

Animal No. (No. of samples) post-occlusion †	PROSTACLANDIN A*		PROSTAGLANDIN E*		PROSTAGLANDIN F*	
	AO	CS	AO	CS	AO	CS
1 (2)	0.75 ± .03	1.07 ± .09	0.31 ± .05	2.10 ± .10	0.80 ± .04	3.00 ± .10
	NS		NS		p <.001	
2 (7)	0.41 ± .02	0.45 ± .02	0.27 ± .01	0.97 ± .08	0.25 ± .01	0.46 ± .02
	NS		p <.001		p <.001	
3 (12)	0.31 ± .01	0.33 ± .02	0.20 ± .01	0.39 ± .01	0.19 ± .01	0.38 ± .03
	NS		p <.001		p <.001	
4 (5)	0.30 ± .02	0.31 ± .02	0.23 ± .02	0.36 ± .06	0.22 ± .02	0.34 ± .02
	NS		p <.05		p <.001	
5 (9)	0.23 ± .01	0.23 ± .02	0.17 ± .02	0.37 ± .04	0.23 ± .02	0.44 ± .07
	NS		p <.001		p <.01	
6 (4)	0.24 ± .03	0.26 ± .04	0.28 ± .02	0.73 ± .08	0.62 ± .04	1.82 ± .03
	NS		p <.05		p <.02	
7 (6)	0.29 ± .01	0.33 ± .02	0.27 ± .04	0.56 ± .02	0.37 ± .02	1.37 ± .06
	NS		p <.02		p <.01	
Mean ± SE ≠	0.30 ± .01	0.33 ± .02	0.24 ± .01	0.44 ± .01	0.26 ± .01	0.67 ± .01
	NS		p <.001		p <.001	

* Plasma prostaglandin concentration, ng./ml. Each value represents the mean of all samples drawn in each animal after occlusion. The sample immediately following occlusion was disregarded (see text).

† Number of samples was determined by the animal's course. The sample drawn immediately after occlusion was not included in these analyses (see text).

≠ Weighted mean determined by weighting individual animal means by the inverse of their variances

AO = Aorta

CS = Coronary sinus

Table 2. Control studies: Prostaglandin levels for individual animal experiments following anesthesia and catheter placement; no coronary occlusion performed.

Animal No.	PROSTAGLANDIN A*		PROSTAGLANDIN E*		PROSTAGLANDIN F*	
(No. of samples)	AO	CS	AO	CS	AO	CS
1 (6)	0.32 ± .01	0.34 ± .01	0.24 ± .01	0.23 ± .02	0.31 ± .01	0.32 ± .02
	NS		NS		NS	
2 (6)	0.36 ± .01	0.35 ± .01	0.22 ± .01	0.24 ± .01	0.30 ± .01	0.29 ± .01
	NS		NS		NS	
Mean ± SE **	0.34 ± .01	0.35 ± .01	0.23 ± .01	0.24 ± .01	0.31 ± .01	0.30 ± .01
	NS		NS		NS	

Ao = Aorta

CS = Coronary sinus

NS = Non-significant

* Plasma prostaglandin concentration, ng./ml.

** Weighted mean determined by weighting individual animal means by the inverse of their variances

Table 3. Regional prostaglandin levels following coronary occlusion.

Animal No. (No. of Samples)† post-occlusion	PROSTAGLANDIN A*			PROSTAGLANDIN E*			PROSTAGLANDIN F*		
	GCV	AO	CS	GCV	AO	CS	GCV	AO	CS
1 (4)	0.39 ± .01	0.37 ± .01	0.38 ± .01	0.60 ± .04	0.20 ± .01	1.03 ± .11	0.30 ± .02	0.26 ± .01	1.53 ± .05
	NS	NS		p <.01	p< .01		NS	p< .001	
2 (4)	0.35 ± .02	0.35 ± .01	0.35 ± .01	0.55 ± .07	0.24 ± .01	1.00 ± .17	0.32 ± .03	0.29 ± .03	1.44 ± .13
	NS	NS		p< .05	p< .05		NS	p< .01	
3 (4)	0.36 ± .01	0.36 ± .01	0.35 ± .02	0.46 ± .03	0.20 ± .01	0.65 ± .08	0.33 ± .03	0.34 ± .01	0.69 ± .11
	NS	NS		p< .01	p <.02		NS	p< .05	
4 (2)	0.51 ± .01	0.48 ± .02	0.50 ± .02	0.57 ± .03	0.23 ± .01	1.18 ± .15	0.41 ± .03	0.33 ± .01	2.06 ± .05
	NS	NS		p< .05	p< .05		NS	p <.02	
5 (2)	0.39 ± .01	0.37 ± .01	0.38 ± .03	0.81 ± .05	0.19 ± .01	1.38 ± .06	0.36 ± .03	0.31 ± .04	1.83 ± .06
	NS	NS		NS	p< .05		NS	p <.05	
6 (5)	0.44 ± .01	0.44 ± .01	0.45 ± .02	0.54 ± .07	0.22 ± .02	0.99 ± .07	0.42 ± .04	0.37 ± .04	1.37 ± .12
	NS	NS		p< .01	p <.001		p <.01	p <.01	
Mean ± SE ≠	0.41 ± .01	0.38 ± .01	0.38 ± .01	0.55 ± .02	0.21 ± .01	1.07 ± .04	0.34 ± .01	0.32 ± .01	1.69 ± .03
	p <.05	NS		p <.001	p <.001		NS	p <.001	
	NS			p< .001			p <.001		

* Plasma prostaglandin concentration, ng./ml. Each value represents the mean of all samples drawn, in each animal, after occlusion. The sample immediately following occlusion was disregarded (see text).

† Number of samples was determined by the animal's course after occlusion. The sample drawn immediately after occlusion was not included for analysis (see text).

≠ Weighted mean determined by weighting individual animal means by the inverse of their variances

AO = Aorta

CS = Coronary Sinus (proximal)

GCV = Great cardiac vein (distal)

NS = Non-significant

Table 4. Clinical and Metabolic Data

Pt. No.	Age (yrs)	Sex	Indication for Catheterization	Prior MI	Significant Coronary Arterial Lesion*			Receiving Propranolol	Peak Atrial Pacing		Lactate Uptake %		PGF (AO-CS)Δ at Peak Pacing
					RCA	LAD	LCF		STΔ	HR	Rest	Pacing	
1	64	M	Angina	-	+	+	+	-	-1.5	150	+21	-23	-0.35
2	47	M	Angina	-	-	+	+	-	-1.8	135	+14	-13	-0.18
3	58	M	Angina	+	+	+	+	+	-1.0	135	+8	-19	-0.50
4	56	M	Angina	-	+	+	-	-	-2.0	150	+10	-1	+0.01
5	53	M	Angina	+	+	+	+	+	-1.5	125	+11	-3	-0.35
6	67	M	Angina	-	+	+	+	+	-1.5	145	+30	-10	-0.45
7	42	M	Angina	-	+	+	+	-	-2.5	150	+41	+6	-0.47
8	52	F	Angina	-	+	+	+	-	-1.2	145	+27	-3	-0.32
9	43	M	Angina	+	+	+	-	+	-1.5	90	+35	-12	-0.30
10	50	M	Angina	-	+	+	-	-	-3.0	120	+37	+5	-0.21
11	44	M	Angina	+	+	+	+	+	-2.0	110	+38	-26	-0.31
12	51	F	Angina	-	-	+	+	-	-1.5	150	+40	-23	-0.21
13	44	M	Atypical chest pain	-	-	-	-	-	0	150	+25	+21	-0.01
14	51	M	Atypical chest pain	-	-	-	-	-	0	160	+26	+22	+0.03
15	46	F	Atypical chest pain	-	-	-	-	-	0	140	+29	+33	+0.01

Abbreviations: (AO-CS)Δ= aorta-coronary sinus difference; HR= heart rate; LAD= left anterior descending coronary artery; LCF= left circumflex coronary artery; MI= myocardial infarction; Pt.No.= patient number; PGF= prostaglandin F concentration (ng/ml); RCA= right coronary artery; STΔ= ST segment change (mm). *+= greater than 75% reduction in luminal diameter

REFERENCES

1. Higgins, C., Braunwald, E.: The prostaglandins - Biochemical, physiologic, and clinical considerations. Amer. J. Med. 53: 92, 1972.
2. Gudbjarnason, S.: Prostaglandins and polyunsaturated fatty acids in heart muscle. J. Mol. Cell. Cardiol. 7:443, 1975.
3. Hamberg, M., Samuelsson, B.: Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. Proc. Nat. Acad. Sci. (USA) 70:899, 1973.
4. Hamberg, M., Svensson, J., Samuelsson, B.: Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Nat. Acad. Sci. (USA) 72:2994, 1975.
5. Ferreira, S., Vane, J.: Prostaglandins: Their disappearance from and release into the circulation. Nature 216:868, 1967.
6. McGiff, J., Terragno, N., Strand, J., et al: Selective passage of prostaglandins across the lung. Nature (London) 223:742, 1969.
7. Horton, E., Jones, R.: Prostaglandins A_1, A_2, 19-hydroxy A_1: Their actions on smooth muscle and their inactivation on passage through the pulmonary and hepatic portal vascular beds. Br. J. Pharmacol. 37:705, 1969.

8. Limas, C., Cohn, J.: Isolation and properties of myocardial prostaglandin synthetase. Cardiovasc. Res. 7:623, 1973.

9. Limas, C., Cohn, J.: Regulation of myocardial prostaglandin dehydrogenase activity. The role of cyclic 3^1, 5^1-AMP and calcium ions. Proc. Soc. Exp. Biol. Med. 142:1230, 1972.

10. Terragno, D., Crawshaw, K., Terragno, N., et al.: Prostaglandin synthesis by bovine mesenteric arteries and veins. Circ. Res. 36/37:I-76, 1975.

11. Gimbrone, M., Alexander, R.: Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium. Science 189:219, 1975.

12. Kulkarni, P., Roberts, R., Needleman, P.: Paradoxical endogenous synthesis of a coronary dilating substance from arachidonate. Prostaglandins 12:337, 1976.

13. Kalsner, S.: Endogenous prostaglandin release contributes directly to coronary artery tone. Canad. J. Physiol. Pharmacol. 53:560, 1975.

14. Klein, I., Levey, G.: Effect of prostaglandins on guinea pig myocardial adenyl cyclase. Metabolism 20:890, 1971.

15. Flower, R.: Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26:33, 1974.

16. Sanner, J.: Substances that inhibit the actions of prostaglandins. Arch. Int. Med. 133:133, 1974.

17. Flower, R., Cheung, H., Cushman, D.: Quantitative determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase (prostaglandin synthetase) system of bovine seminal vesicle. Prostaglandins 4:325, 1973.

18. Moncada, S., Needleman, P., Bunting, S., et al.: Prostaglandin endoperoxide and thromboxane generating systems and their selective inhibition. Prostaglandins 12:323, 1976.

19. Crutchley, D., Piper, P.: Inhibition of the inactivation of prostaglandins in guinea pig lungs. Arch. Pharmacol. 279: Suppl R, 20, 1973.

20. Sanner, J., Eakins, K.: Prostaglandin antogonists. In Prostaglandins: Chemical and biochemical aspects (Karim, S., Ed.), New York, 1976, p. 139.

21. Rowe, G., Afonso, S.: Systemic and coronary hemodynamic effects of intracoronary administration of prostaglandin E_1 and E_2. Amer. Ht. J. 88:51, 1974.

22. Hollenberg, M., Walker, R., McCormick, D.: Cardiovascular responses to intracoronary infusion of prostaglandin E_1,$F_{1\alpha}$,$F_{2\alpha}$. Arch. Int. Pharmacodyn. 174:66, 1968.

23. Nakano, J.: Effects of prostaglandins E_1, A_1, and $F_{2\alpha}$ on the coronary and peripheral circulations. Proc. Soc. Exper. Ther. 127:1160, 1968.

24. Bloor, C., White, F., Sobel, B.: Coronary and systemic hemodynamic effects of prostaglandins in the unanesthetized dog. Cardiovasc. Res. 7:156, 1973.

25. Su, J., Higgins, C., Friedman, W.: Chronotropic and inotropic effects of prostaglandins E_1, A_1, and $F_{2\alpha}$ on isolated mammalian cardiac tissue. Proc. Soc. Exp. Biol. Med. 143:1227, 1973.

26. Nutter, D., Ratts, T.: Direct actions of prostaglandins E_1, A_1, and $F_{2\alpha}$ on myocardial contraction. Prostaglandins 3: 323, 1973.

27. Higgins, C., Vatner, S., Franklin, D., Braunwald, E.: Effects of prostaglandin A_1 on left ventricular dynamics in the conscious dog. Amer. J. Physiol. 222:1534, 1972.

28. DuCharme, D., Weeks, J., Montgomery, R.: Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$. J. Pharmacol. Exp. Ther. 160:1, 1968.

29. Brody, M., Kadowitz, P.: Prostaglandins as modulators of the autonomic nervous system. Fed. Proc. 33:48, 1974.

30. Hutton, I., Parratt, J., Lawrie, T.: Cardiovascular effects of prostaglandin E_1 in experimental myocardial infarction. Cardiovasc. Res. 7:149, 1973.

31. Goldfarb, R., Glenn, T.: Beneficial effects of prostaglandin $F_{2\alpha}$ in experimental myocardial infarction. Amer. J. Cardiol. 33:139, 1974.

32. Raflo, G., Wangensteen, S., Glenn, T., et al: Mechanism of the protective effects of prostaglandins E_1 and $F_{2\alpha}$ in the canine endotoxin shock. Euro. J. Pharm. 24:86, 1973.

33. Gottwik, M., Kirk, E., Hoffstein, S., et al.: Effect of collateral flow on epicardial and endocardial lysosomal hydrolases in acute myocardial ischemia. J. Clin. Invest. 56:914, 1975.

34. Reynolds, R., Kelleher, G., Roberts, J.: Effect of prostaglandin E_1 on coronary occlusion-induced arrhythmia. Fed. Proc. 34:763, 1975.

35. Riley, R., Polis, D., Angelakos, E.: Protective effect of a prostaglandin derivative on mortality following coronary ligation and ventricular fibrillation. Physiologist 17: 320, 1974.

36. McGiff, J., Crawshaw, K., Terragno, N., et al: Prostaglandin-like substances appearing in canine renal venous blood during renal ischemia. Circ. Res. 27:765, 1970.

37. Herbaczynska-Cedro, K., Vane, J.: Prostaglandins as mediators of reactive hyperemia in kidney. Nature 247:492, 1974.

38. Pickard, T., MacKenzie, E.: Inhibition of prostaglandin synthesis and the response of baboon cerebral circulation to carbon dioxide. Nature 245:187, 1973.

39. Franklin, G., Dowd, A., Caldwell, B., et al: The effect of angiotensin-II intravenous infusion on plasma renin activity and prostaglandins A, E, and F levels in the uterine vein of the pregnant monkey. Prostaglandins 10:200, 1975.

40. Wenmalm, A., Pham-Huu-Chanh, Junstad, M.: Hypoxia causes prostaglandin release from perfused rabbit hearts. Acta Physiol. Scand. 91:133, 1974.

41. Wenmalm, A.: Prostaglandin release and mechanical performance in the isolated rabbit heart during induced changes in the internal environment. Acta Physiol. Scand. 93:15, 1975.

42. Junstad, M., Wenmalm, A.: Release of prostaglandin from the rabbit isolated heart following vagal nerve stimulation or acetylcholine infusion. Br. J. Pharmacol. 52:375, 1974.

43. Needleman, P., Minkes, M., Douglas, J.: Stimulation of prostaglandin biosynthesis by adenine nucleotides. Circ. Res. 34:455, 1974.

44. Block, A., Feinberg, H., Herbaczynska-Cedro, K., et al: Anoxia-induced release of prostaglandins in rabbit isolated hearts. Circ. Res. 36:34, 1975.

45. Block, A., Poole, S., Vane, J.: Modification of basal release of prostaglandins from rabbit isolated hearts. Prostaglandins 7:473, 1974.

46. Alexander, R., Kent, K., Pisano, J., et al: Regulation of postocclusive hyperemia by endogenously synthesized prostaglandins in the dog heart. J. Clin. Invest. 55:1174, 1975.

47. Kraemer, R., Folts, J.: Release of prostaglandin following temporary occlusion of the coronary artery. Fed. Proc. 32: 454, 1973.

48. Afonso, S., Bandow, G., Rowe, G.: Indomethacin and the prostaglandin hypothesis of coronary blood flow regulation. J. Physiol. 241:299, 1974.

49. Owen, T., Ehrhart, I., Chen, W., et al: Failure of indomethacin to alter reactive hyperemia in the dog heart. Physiologist 16:413, 1973.

50. Rubio, R., Berne, R.: Regulation of coronary blood flow. Prog. Cardiovasc. Dis. 18:105, 1975.

51. Ogletree, M., Lefer, A.: Influence of nonsteroidal anti-inflammatory agents on myocardial ischemia in the cat. J. Pharmacol. Exper. Ther. 197:582, 1976.

52. Ung, S., Johnson, A., LeWinter, M., et al: The effects of acetyl salicylic acid on ischemic injury following coronary occlusion in the open chest dog. Clin. Res. 24:90A, 1976.

53. Widemeir, V., Logan, M.: Effects of indomethacin, polyphloretin phosphate, and SC19220 on coronary vascular responses to occlusion, adenosine, and PGE_1. Red. Proc. 33:489, 1974.

54. Samuelsson, B., Granstrom, E., Green, K., et al: Prostaglandins. Ann. Rev. Biochem. 44:669, 1975.

55. Needleman, P., Moncada, S., Bunting, S., et al: Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides. Nature 261:558, 1976.

56. Ellis, E., Oelz, O., Roberts, L., et al: Coronary arterial smooth muscle contraction by a substance released from platelets: Evidence that it is a thromboxane A_2. Science 193:1135, 1976.

57. Splawinski, J., Nies, A., Sweetman, B., et al: The effects of arachidonic acid, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ on the longitudinal stomach strip of the rat. J. Pharm. Exper. Ther. 187:501, 1973.

58. Mustard, J.: Platelets and thrombi in acute myocardial infarction. Hosp. Pract. 7:115, 1972.

59. Elwood, P., Cochrane, A., Burr, M., et al: A randomized controlled trial of acetyl salicylic acid in the secondary prevention of mortality from myocardial infarction. Brit. Med. J. 9 Mar 1974 p. 436.

60. Boston Collaborative Drug Surveillance Group: Regular aspirin intake and acute myocardial infarction. Brit. Med. J. 9 Mar 1974 p. 440.

61. Frishman, W., Christadoulou, J., Weksler, B., et al: Aspirin therapy in angina pectoris: Effects on platelet aggregation, exercise tolerance, and electrocardiographic manifestations of ischemia. Amer. Ht. J. 92:3, 1976.

62. Scheuer, J.: Myocardial metabolism in cardiac hypoxia. Amer. J. Cardiol. 19:384, 1967.

63. Katz, A., Hecht, H.: The early pump failure of the ischemic heart. Amer. J. Med. 47:497, 1969.

64. Opie, L.: Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Amer. J. Cardiol. 36:938, 1975.

65. Kjekshus, J., Mjøs, O.: Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart. J. Clin. Invest. 51:1767, 1972.

66. Kadowitz, P., McNamara, D., Chapnick, B., et al: Influence of arachidonic acid on the canine coronary vascular bed. Circ. 54:II-107, 1976.

67. Nakazawa, H., Oliveros, R., Orlick, A., et al: Evaluation of regional variations in coronary flow by simultaneous great cardiac vein and coronary sinus sampling. Circ. 49/50:III-140, 1974.

68. Snedecor, G., Cochran, W.: Statistical Methods, Sixth Edition. Ames, Iowa, Iowa State University Press, 1973, pp. 91-119, 436-438.

69. Krasnow, N., Neill, N., Messer, J., et al: Myocardial lactate and pyruvate metabolism. J. Clin. Invest. 41: 2075, 1962.

70. Parker, J., Chiong, M., West, R., et al: Sequential alterations in myocardial lactate metabolism, S-T segments, and left ventricular function during angina induced by atrial pacing. Circ. 40:113, 1969.

71. Herman, M., Elliott, W., Gorlin, R.: An electrocardiographic, anatomic and metabolic study of zonal myocardial ischemia in coronary heart disease. Circ. 35:834, 1967.

72. Auletta, F., Zusman, R., Caldwell, B.: Development and standardization of radioimmunoassays for prostaglandins E, F and A. Clin. Chem. 20:1580, 1974.

73. Stylos, W., Howard, L., Ritzi, E., et al: The preparation and characterization of prostaglandin E_1 antiserum. Prostaglandins 6:1, 1974.

74. Maroko, P., Kjekshus, J., Sobel, B., et al: Factors influencing infarct size following coronary artery occlusion. Circ. 43:67, 1971

75. Jones, R., Cammock, S., Horton, E.: Partial purification and properties of cat plasma prostaglandin isomerase. Biochemica Biophysica Acta 280:588, 1972.

76. Wagner, S., Terragno, N., Terragno, D., et al: Modulation of prostaglandin synthesis by an NADPH dependent enzyme in rabbit kidney. Clin. Res. 22:627, 1974.

77. Limas, C., Cohen, J.: Stimulation of vascular smooth muscle sodium, potassium-adenosinetriphosphatase by vasodilators. Circ. Res. 35:601, 1974.

78. Lawery, H., Lowe, R., Scroop, G.: Central autonomic effects of prostaglandin $F_{2\alpha}$ on the cardiovascular system of the dog. Br. J. Pharmacol. 41:454, 1971.

79. Jaffe, B., Behrman, H., Parker, C.: Radioimmunoassay measurement of prostaglandins E, A and F in human plasma. J. Clin. Invest. 52:398, 1973.

80. Angell, C., Falsetti, G., Weisfeldt, M., et al: Relationship of intramyocardial oxygen tension and epicardial ST segment changes following acute coronary artery ligation: effects of coronary perfusion pressure. Cardiovasc. Res. 9:12, 1975.

81. Stephen, K., Mesman, W., Sadony, V.: Oxygen demand and collateral vessels of the heart: Factors influencing the severity of myocardial ischemic injury after experimental coronary occlusion. Cardiovasc. Res. 9:640, 1975.

82. Smith, H., Singh, B., Norris, R., et al: Changes in myocardial blood flow and ST segment elevation following coronary artery occlusion in dogs. Circ. Res. 36:697, 1975.

83. Kjekshus, J., Maroko, P., Sobel, B.: Distribution of myocardial injury and its relation to epicardial ST segment changes after coronary artery occlusion in the dog. Cardiovasc. Res. 6:490, 1972.

84. Buckberg, G., Tuck, J., Payne, B., et al: Some sources of error in measuring regional blood flow with radioactive microspheres. J. Appl. Physiol. 31:598, 1971

85. Ross, R.: Introduction to the problem of myocardial ischemia. In Myocardial Ischemia. (Ross, R., ed.) New York, Excerpta Medica, 1971, pp. 1-6.

86. Sonnenblick, E., Skelton, C.: Oxygen consumption of the heart: physiological principles and clinical implications. Mod. Conc. Cardiovasc. Dis. 40:9, 1971.

87. Parker, J., Chiong, M., West, R., et al: The effect of ischemia and alterations of heart rate on myocardial potassium balance in man. Circ. 42:205, 1970.

88. Fox, A., Reed, G., Glassman, E., et al: Release of adenosine from human hearts during angina induced by rapid atrial pacing. J. Clin. Invest. 53:1447, 1974.

89. Pitt, B., Mason, J., Conti, C., et al: Activation of the plasma kallikrein system during myocardial ischemia. In Advances in Experimental Medicine and Biology. Vol. 8. (Secuteri, F., Rocha, M., Silva, E., Back, N., eds.) New York, Plenum Press, 1970, pp. 403-410.